201-14686

# The Flavor and Fragrance High Production Volume Consortia (FFHPVC)

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August 19, 2003

Christie Todd Whitman, Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Ms. Whitman:

On behalf or the member companies of the Cyclohexyl Derivatives Consortium, the Flavor and Fragrance High Production Volume Consortia is pleased to submit the Test Plan and Robust Summaries for the chemical category designated the "Alkyl-substituted Cyclohexanol Derivatives" to the HPV Challenge Program, AR-201. The Cyclohexyl Derivatives Consortium has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public.

This submission includes one electronic copy in .pdf format. Hard copy can be provided upon request. The EPA registration number for the Cyclohexyl Derivatives Consortium is

Please feel free to contact me with any questions or comments you might have concerning the submission at tadams@therobertsgroup.net, tadams@chemintox.com or 202-331-2325.

Sincerely,

Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

# The Flavor and Fragrance High Production Volume Consortia

# The Cyclohexyl Derivatives Consortium

# Test Plan for Alkyl-substituted Cyclohexanol Derivatives

**4-*tert*-butylcyclohexanol** **CAS No. 98-52-2**

**4-*tert*-butylcyclohexyl acetate** **CAS No. 32210-23-4**

## FFHPVC Cyclohexyl Derivatives Consortium Registration Number

**Submitted to the EPA under the HPV Challenge Program by:**

**The Flavor and Fragrance High Production Volume Chemical Consortia**

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**Washington, DC 20006**

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# List of Member Companies

**FIRMENICH, INC.**

**INTERNATIONAL FLAVORS & FRAGRANCES INC.**

**J. MANHEIMER, INC.**

**QUEST INTERNATIONAL**

**DEGUSSA AG/DEGUSSA CORPORATION**

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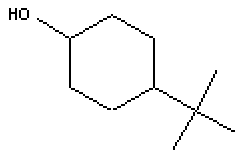
# Test Plan for Alkyl-substituted Cyclohexanol Derivatives

## 1 IDENTITY OF SUBSTANCES

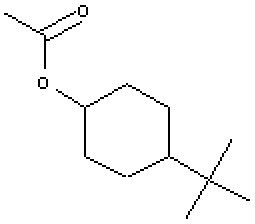



**4-*tert*-Butylcyclohexanol**

**CAS NO. 98-52-2**

***Synonyms:***

**Cyclohexanol, 4-(1,1-dimethylethyl)-**




**4-*tert*-Butylcyclohexyl acetate**

**CAS NO. 32210-23-4**

***Synonyms:***

**Acetic acid, *p-tert*-butylcyclohexyl ester**

**4-*tert*-Butylhexahydrophenyl acetate**

# 2 CATEGORY ANALYSIS

## 2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The cyclohexyl consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for cyclohexyl substances under the Chemical Right-to-Know Program. Five (5) companies are current members of the Cyclohexyl Consortium. The Cyclohexyl Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

## 2.2 BACKGROUND INFORMATION

4-*tert*-Butylcyclohexanol and its corresponding acetate ester, 4-*tert*-butylcyclohexyl acetate are used primarily in soap perfumes. Both substances exist in *cis* and *trans* forms. *trans*-4-*tert*-Butylcyclohexyl acetate exhibits a strong woody aroma while *cis*-4-*tert*-butylcyclohexyl acetate is a more intense woody aroma with a flowery note. The ester has far greater use as a fragrance than does the corresponding alcohol. The alcohol serves as a synthetic precursor of the acetate. Wide variation in the ratio of the *cis* and *trans* isomers does not significantly alter the physical properties. A mixture of *cis* and *trans* alcohol is prepared by hydrogenation of 4-*tert*-butylphenol. The acetate is obtained by acetylation of the *cis* and *trans* mixture of the alcohol.

## 2.3 STRUCTURAL CLASSIFICATION

This category consists of 2 substances, 4-*tert*-butylcyclohexanol and its corresponding acetate ester, 4-*tert*-butylcyclohexyl acetate. Safety data on the structurally related alkyl-substituted cyclohexanol and cyclohexanone derivatives (*e.g.*, 2-isopropyl-5-methylcyclohexanol, 2-, 3-, and 4-*tert*-butylcyclohexanone and 2-, 3-, and 4-methylcyclohexanone) are also included in this chemical category.

## 2.4 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

### 2.4.1 Hydrolysis of Esters of Cyclohexanol

The unsubstituted monocyclic esters (*e.g.* cyclohexyl acetate) are rapidly hydrolyzed to cyclohexanol and the component aliphatic carboxylic acids by classes of enzymes recognized as carboxylesterases [White *et al.*, 1990; Ford and Moran, 1978; Heymann, 1980], the most important of which are the *beta*-esterases. In mammals, these enzymes occur in most tissues [Anders, 1989; Heymann, 1980] but predominate in the hepatocytes [Heymann, 1980].

*Cis*- and *trans*-1-methylene-4-isopropenylcyclohexan-2-yl acetate is rapidly hydrolyzed *in vitro* in the presence of rat liver homogenate [Salzer, 1998]. Incubation of the ester resulted in 92% hydrolysis after 15 minutes and 100% after 60 minutes [Salzer, 1998]. The structurally related ethylene glycol and propylene glycol carbonate esters of (-)-2-isopropyl-5-methylcyclohexanol are completely hydrolyzed after incubation for 1 hour with rat liver homogenate [Emberger, 1994]. Sterically hindered esters of cyclohexanol are also readily hydrolyzed in rat liver homogenate. The ester, 3,5,5-trimethyl-[2,3-$^{3}$H]-cyclohexanyl-[$^{14}$C]-mandelate (cyclandelate), was completely hydrolyzed to 3,5,5-trimethyl-[2,3-$^{3}$H]-cyclohexanol and [$^{14}$C]-mandelic acid within 5 minutes of incubation with microsomal rat hepatocytes. After 20 minutes, 80% of the alcohol disappeared while there was a concomitant linear increase in a *beta*-glucuronidase reactive substance [White *et al.*, 1990]. Presumably, the resulting alcohol was conjugated with glucuronic acid.

Urine collected 18 hours post administration of 350 mg/kg bw of the acetate ester of 2-cyclohexen-1-ol (*i.e.*, cyclohex-1-en-1-yl acetate) to rabbits revealed that 39% of the dose was hydrolyzed and then conjugated with glucuronic acid [Elliott *et al.*, 1959]. Based on this information, it is anticipated that 4-*tert*-butylcyclohexyl acetate is hydrolyzed *in vivo* to yield 4-*tert*-butylcyclohexanol and acetic acid.

Once formed, cyclohexanol or alkyl-substituted cyclohexanols are rapidly absorbed through the gastrointestinal tract and rapidly eliminated from the blood. Peak blood levels are normally reached within 1-2 hours after dosing. Unsubstituted or alkyl-substituted cyclohexanol is rapidly oxidized *in vivo* to the corresponding cyclohexanone derivative by alcohol dehydrogenase. Conversely, the cyclohexanone derivative may be reduced to cyclohexanol by cytosolic carbonyl reductases. Hence, the ketone and alcohol are interconvertible *in vivo*. Conjugation of the alcohol with glucuronic acid and excretion in the bile and urine provides the predominant pathway for metabolic detoxication and elimination of cyclohexanol. Also, since cyclohexanone is readily converted to cyclohexanol and then the glucuronic acid conjugate of cyclohexanol *in vivo*, data on cyclohexanone derivatives are directly relevant to the hazard assessment of cyclohexanol derivatives.

Male Sprague-Dawley rats were exposed to atmospheres of either 400 ppm (240 mg/kg bw) or 1600 ppm (980 mg/kg bw) cyclohexanone for 6 hours. Twenty-four hour post-exposure terminal blood and urine samples show the average plasma levels of cyclohexanone and cyclohexanol for the 400 ppm and 1600 ppm exposures were 26 and 20 micrograms/ml and 122 and 140 micrograms/ml, respectively. The total urinary excretion of cyclohexanol was at least 10 times that of cyclohexanone (16 and 15 micrograms and 143 and 264 micrograms at the 400 and 1600 ppm exposures, respectively) with 13 micrograms and 72 micrograms of conjugated cyclohexanol being excreted within 72 hours at 400 ppm and 1600 ppm, respectively [Topping *et al*., 1994].

In another study, four rabbits were each given cyclohexanone (No. 1100) in water by gavage. Urine collected at 18 hours after dosing revealed 66% of the 248 mg/kg oral dose

was excreted as the glucuronic acid conjugate of cyclohexanol [Elliott *et al.*, 1959]. The authors concluded that cyclohexanone is first reduced to cyclohexanol and then conjugated with glucuronic acid prior to excretion in the urine.

Male beagle dogs were given 284 mg/kg bw of cyclohexanone by intravenous injection daily. Cyclohexanol was detected in the plasma within 30 minutes of injection. The mean distribution and elimination half-lives of cyclohexanone and cyclohexanol are 6.6 and 81 minutes, respectively. The mean steady state volume of distribution for cyclohexanone is 2.6 L/kg and the mean total body clearance for cyclohexanone is 27.4 ml/kg/minutes. [Martis *et al.*, 1980; Koeferl *et al.*, 1981]. When 328 mg/kg bw cyclohexanol was administered by intravenous injection, it showed a plasma half-life of 99 minutes, an apparent distribution volume of 1.2 L/kg and a total body clearance of 8.8 ml/kg/minutes. Based on these data cyclohexanone and cyclohexanol are rapidly cleared from the body [Martis *et al.*, 1980]. Approximately 60% of cyclohexanone administered was recovered in the urine as a glucuronide conjugate of cyclohexanol after 24 hours. The direct renal clearance of unmodified cyclohexanone and cyclohexanol is a minor route of elimination accounting for less than 1% of the administered dose. It is proposed that 74-100% of cyclohexanone is converted to cyclohexanol and further metabolized before elimination. The authors propose that some of the cyclohexanone may be expelled through the lungs [Martis *et al.*, 1980; Koeferl *et al.*, 1981].

Four men and four women volunteers were exposed to an environment containing atmospheric concentration of 101, 207, or 406 mg/cu.m of cyclohexanone for eight (8) hours. Urine collected at 2-hour intervals during exposure and for 72 hours post-exposure show the presence of glucuronic acid conjugates of cyclohexanediol with peak excretion rate at about 16 hours post-exposure. Approximately 60% of the cyclohexanone dose is excreted within the 72-hour period [Mraz *et al.*, 1994].

An adult man ingested 100 ml of liquid adhesive containing 39% cyclohexanone. The cyclohexanone was rapidly absorbed. Plasma and urine levels of cyclohexanone and metabolites were unaffected by gastric lavage (5.5 L saline), two plasma exchanges (2.4 L each) and hemoperfusion when compared to pre-treatment values. Cyclohexanol and

cyclohexanone were detected in the plasma for up to 25 hours post ingestion. Cyclohexanone levels were at the lower limit of detection, however, plasma levels of cyclohexanol were high, 220 micrograms/ml 5 hours after ingestion and decreased to 10 micrograms/ml after 20 hours. High levels of cyclohexanol glucuronide were detected in the urine for up to 48 hours. Urinary excretion of the parent ketone was described as minimal. The elimination half-life of cyclohexanone in human plasma was determined at 4.75 hours and the rate of elimination ($K_e$) 0.145 micrograms/ml/hour. This indicates that the mechanism of elimination in humans involves conversion of the cyclohexanone to cyclohexanol followed by conjugation with glucuronic acid [Sakata *et al.*, 1989].

Other unsubstituted alicyclic ketones (e.g., cyclopentanone) are rapidly absorbed, metabolized, conjugated and excreted mainly in the urine [James and Waring, 1971]. The urine collected from rabbits orally administered 193 mg/kg bw cyclopentanone was taken and treated with *beta*-glucuronidase. The resulting analysis revealed that the major urinary component was a glucuronic acid conjugate of cyclopentanol [James and Waring, 1971].

The size, position, number, or stereochemistry of alkyl substituents on the cyclohexyl ring exerts no significant effect on the rate of absorption, metabolism and excretion of alkyl-substituted cyclohexanol or cyclohexanone derivatives. Alkyl-substituted cyclohexanols are rapidly absorbed, conjugated with glucuronic acid, and excreted mainly in the urine. Alkyl-substituted cyclohexanones are also rapidly absorbed, reduced to the corresponding cyclohexanol derivatives that are then conjugated with glucuronic acid and also excreted mainly in the urine.

The urine of groups (6 to 10) of doe albino rabbits was pooled 24 hours after each animal received a single oral dose of 652 mg/kg bw of (±)-2-*tert*-butylcyclohexanone, 652 mg/kg bw of (±)-3-*tert*-butylcyclohexanone, or 562 mg/kg bw of 4-*tert*-butylcyclohexanone [Cheo *et al*., 1967]. The mean % of the dose excreted as the glucuronic acid is 76.5, 90, or 80% respectively.

Rabbits given oral doses of 1750 mg/kg bw of methylcyclohexanol (mixture of isomers) or 560 mg/kg bw of methylcyclohexanone (mixture of isomers) predominantly excrete the glucuronic acid of methylcyclohexanol within the first 24 hours [Treon *et al.*, 1943a]. Rabbits were exposed to atmospheres containing 2.3 (503 ppm), 1.06 (232 ppm), or 0.56 mg/L (121 ppm) of methylcyclohexanol (mixture of isomers), 6 hours daily, 5 days per week for 10 weeks. Mean daily urinary output of glucuronic acid conjugates during exposure is proportional to dose. Rabbits exposed to atmospheres containing 2.31 (514 ppm) or 0.816 mg/L (132 ppm) of methylcyclohexanone (mixture of isomers), 6 hours daily, 5 days per week for 10 weeks exhibit mean daily urinary output of glucuronic acid proportional to dose [Treon *et al.*, 1943b].

Rats received 500 mg/kg bw (128 microcurie/mg) of 3-$^{3}$H-2-isopropyl-5-methylcyclohexanol and urine and feces were collected 24 and 48 hours after dosing. The total excretion of 3-$^{3}$H-2-isopropyl-5-methylcyclohexanol by intact and bile duct-cannulated rats was greater than 70% of the dose at 48 hours. The glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol and other minor oxidized metabolites are present in urine and fecal extracts. The glucuronic acid conjugate is also the main metabolite in the bile, while the glucuronic acid conjugate and minor metabolites (less than 5%) formed by side-chain oxidation are excreted in the urine [Yamaguchi *et al.*, 1994].

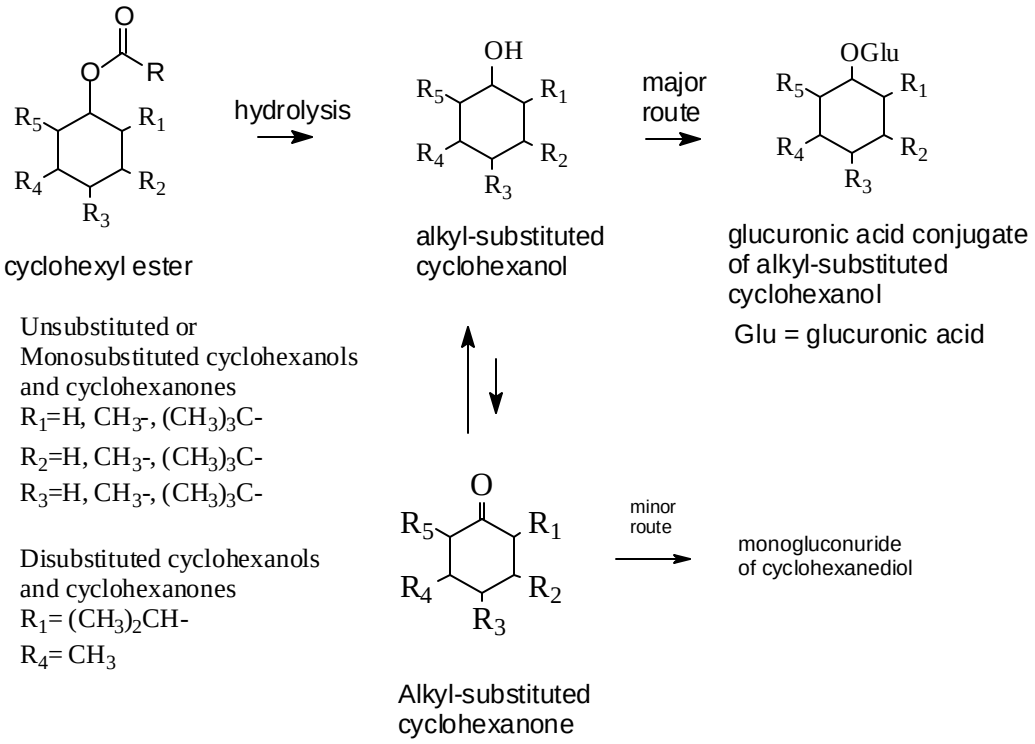
In summary, the esters of cyclohexanol are readily hydrolyzed. In the principal excretion pathway, the cyclohexanols are conjugated with glucuronic acid and excreted primarily in the urine. Also, since alkyl-substituted cyclohexanols are interconvertible with their corresponding ketones *in vivo*, data on alkyl-substituted cyclohexanones are relevant to the evaluation of the 4-*tert*-butylcyclohexanol and its corresponding acetate ester, 4-*tert*-butylcyclohexyl acetate as well.

### 2.4.2 Metabolism

As indicated above, 4-*tert*-butylcyclohexyl acetate will undergo hydrolysis to yield 4-*tert*-butylcyclohexanol. Subsequently 4-*tert*-butylcyclohexanol is conjugated with glucuronic acid to yielded the corresponding glucuronide that is excreted mainly in the urine. This metabolic pathway can be derived from studies with cyclohexanone derivatives. The major metabolic pathway involves reduction of the cyclohexanones to yield the corresponding cyclohexanols that are subsequently excreted primarily as the glucuronic acid conjugates [Lington and Bevan, 1994; Topping *et al*., 1994; Cheo *et al*., 1967; Elliot *et al*., 1965; Yamaguchi *et al.*, 1994]. To a very minor extent, alicyclic ketones and secondary alcohols containing an alkyl side-chain undergo oxidation of the side-chain to form polar poly-oxygenated metabolites that are also excreted as the glucuronide or sulfate conjugates mainly in the urine.

Although it has been anticipated that lipophilic alcohols or ketones with sterically hindered functional groups would undergo more extensive oxidation of alkyl ring substituents [Nelson *et al.*, 1992], studies with 2-, 3-, or 4-methylcyclohexanol, 2-isopropyl-5-methylcyclohexanol, 3,5,5-trimethylcyclohexanol, and even 2-, 3-, or 4-*tert*-butyl-substituted cyclohexanol or cyclohexanones reveal that conjugation of the cyclohexanol moiety by glucuronic acid is the predominant excretion pathway regardless of the size or position of the ring substituent. In general, the metabolic fate of alkyl-substituted cyclohexanol and cyclohexanone derivatives is similar to that of the un-substituted homologues (see Figure 1) [Lington and Bevan, 1994; Topping *et al*, 1994].

## FIGURE 1. METABOLIC FATE OF CYCLOHEXYL DERIVATIVES IN ANIMALS




In rats and rabbits, 66% of a 186 mg/kg bw dose of cyclohexanone or 47% of a 193 mg/kg bw dose of cyclopentanone *via* gavage is reduced to the corresponding secondary alcohol and excreted in the urine as the glucuronic acid conjugate [James and Waring, 1971]. Also, detected are trace amounts of mercapturic acid conjugate of the 2-hydroxycyclohexyl derivative [James and Waring, 1971]. Eighteen (18)-hour urine samples from rabbits administered 1500 mg of cyclohexanone by gavage contain 65% cyclohexanol and a minor amount (6%) of *trans*-cyclohexane-1,2-diol as

monoglucuronide conjugates [Elliott *et al.*, 1959]. Presumably, the diol forms by hydroxylation at the *alpha*-position of cyclohexanone followed by reduction of ketone function. The corresponding cyclohexanol derivative is the major urinary metabolite obtained from rabbits fed 460 mg/kg bw cyclohexane, 260 mg/kg bw cyclohexanol, or 350 mg/kg bw cyclohex-1-en-1-yl acetate [Elliott *et al.*, 1959].

The urine of rabbits given an oral dose of 1200 mg/kg bw of cyclohexanol, shows a significant increase in glucuronic acid conjugates and decrease in inorganic sulfate compared to pre-dose levels [Treon *et al.*, 1943a]. The glucuronic acid conjugate of cyclohexanol is also obtained as the major urinary metabolite in rabbits given 890 mg/kg bw of cyclohexanone [Treon *et al*., 1943a]. The glucuronic acid conjugate of cyclohexanol (1.55 mg/L) and small amounts of cyclohexanone (0.23 mg/L) were found in the urine of workers occupationally exposed to a mixture of atmospheric hexanes including 456 mg/cu.m of cyclohexane [Governa *et al.*, 1987; Perbellini *et al.*, 1980]. The authors concluded that the cyclohexane is transformed to cyclohexanol that subsequently forms glucuronic acid and sulfate conjugates.

Rats and rabbits were given oral doses of 200 - 3200 mg/kg bw of 2-, 3-, or 4-methylcyclohexanone. The glucuronic acid and sulfate conjugates of the corresponding secondary alcohols were the predominant urinary metabolites [Treon *et al.*, 1943a; Elliott *et al.*, 1959; Tao and Elliott, 1962].

Although the glucuronic acid conjugation of the alcohol is the predominant excretion pathway, oxidation of the alkyl substituents to yield poly-oxygenated metabolites has been reported as a minor pathway in animals. The number of possible polyoxygenated metabolites increases with an increase in the types of alkyl ring substituents (*e.g*., methyl and isopropyl substituents) [Nelson *et al*., 1992; Yamaguchi *et al*., 1994; Madyastha and Srivatsan, 1988; Asakawa *et al*., 1986].

The glucuronic acid conjugate of 2-, 3-, or 4-*tert*-butylcyclohexanol is the major urinary metabolite obtained 24 hours after rabbits were given 652 mg/kg bw of (±)-2-*tert*-butylcyclohexanone, 652 mg/kg bw of (±)-3-*tert*-butylcyclohexanone, or 562 mg/kg bw

of 4-*tert*-butylcyclohexanone, respectively [Cheo *et al.*, 1967]. The mean percent of dose excreted is 76.5, 90, or 80% for 2-, 3-, or 4-*tert*-butylcyclohexanone, respectively. The ratio of *cis*- to *trans*-*tert*-butylcyclohexanol present in the urine of animals given 2-(71:29), 3-(74:26), or 4(26:74)-*tert*-butylcyclohexanone provides evidence that carbonyl reductase catalyzed reduction of the ketone function with NADH is influenced by steric effects of the *tert*-butyl substituent. The authors suggest that NADH uses a perpendicular approach to the carbonyl function in 2- and 3-*tert*-butylcyclohexanone. The 4-*tert*-butyl substituent, being more removed from the reaction site, exerts only a minor impact on stereochemistry of the reduction of the ketone to the alcohol. In contrast, a "face to face" approach is used during the reduction of the corresponding smaller alkyl substituents (*e.g.*, methyl-substituted cyclohexanones) by NADH. In these cases, the *trans* isomer is favored [Elliott *et al.*, 1965].

The presence of multiple alkyl substituents at different positions on the cyclohexyl ring does not significantly alter the principal pathway of metabolism and excretion. 2-Isopropyl-5-methylcyclohexanol is mainly conjugated with glucuronic acid. At higher dose levels, *omega*-oxidation of the side chain substituents occurs to yield various polyols and hydroxyacids of 2-isopropyl-5-methylcyclohexanol [Yamaguchi *et al.*, 1994; Madyastha and Srivatsan, 1988]. The unchanged alcohol and minor metabolites formed by side chain oxidation are eliminated in the urine and feces either unchanged or conjugated with glucuronic acid [Yamaguchi *et al.*, 1994]. The corresponding ketone is primarily reduced to the corresponding secondary alcohol that is then eliminated as noted above [Williams, 1940].

The metabolic fate of 2-isopropyl-5-methylcyclohexanol and 2-isopropyl-5-methylcyclohexanone has been studied in humans and other animals. Seventy-nine percent (79%) of a 1000 mg oral dose [Quick, 1928] or 78% of a 10-20 mg oral dose [Atzl *et al.*, 1972] of 2-isopropyl-5-methylcyclohexanol administered to volunteers is eliminated as the glucuronic acid conjugate. For eight days, 750 mg of the *l* stereoisomer of 2-isopropyl-5-methylcyclohexanol was orally administered to three human volunteers followed by oral or intravenous administration of 200 mg [6-$^{13}$C]-glucuronolactone or [6-

$^{13}$C]-sodium glucuronate. Up to 84% of the administered dose of labeled 2-isopropyl-5-methylcyclohexanol is excreted as the glucuronic acid conjugate in the urine after 48 hours [Eisenberg *et al*., 1955]. In two separate studies involving a total of 19 male and female volunteers, the glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol is detected in the urine following oral administration of a 180 mg dose of an essential oil (peppermint oil) containing greater than 80% of 2-isopropyl-5-methylcyclohexanol, its stereoisomers, and the corresponding ketone [Kaffenberger and Doyle, 1990]. A 4500 mg/kg bw oral dose of 2-isopropyl-5-methylcyclohexanol administered to rabbits is conjugated with glucuronic acid and eliminated in the urine [Deichmann and Thomas, 1943; Williams, 1939; Quick, 1924].

In rats, the vast majority of orally administered 2-isopropyl-5-methylcyclohexanol is eliminated in either the urine or feces as the glucuronic acid conjugate or, to a lesser extent, as various oxidation products of the alcohol [Yamaguchi *et al*., 1994; Madyastha and Srivatsan, 1988]. Non-cannulated and bile duct-cannulated male Fischer 344 rats (5/sex) were administered a single dose of 500 mg [3-$^{3}$H]-*l*-2-isopropyl-5-methylcyclohexanol/kg bw. Urine and feces were collected over the next 24 and 48 hours in non-cannulated rats. In the bile duct-cannulated rats, three bile samples were collected in two-hour intervals for the first six hours and a final sample was collected after 24 hours. Urine was collected at 24 hours.

In the non-cannulated rats, total recovery of the labeled substance in the urine or feces is 71.7% with the majority of the dose (45.4%) being recovered within the first 24 hours. In the urine, 37.8% percent of the radioactivity is excreted with equal amounts for the first and second 24 hours. In the feces, 33.9% of the radioactivity is recovered with the majority in the first 24 hours (26.6%) [Yamaguchi *et al.*, 1994]. In the bile duct-cannulated rats, total recovery of the labeled substance in the urine or bile is 74.2% with the majority being recovered in the bile (66.9%). The bile metabolites are mainly the glucuronic acid conjugate of 2-isopropyl-5-methylcyclohexanol along with a variety of oxidation products in which the alkyl substituents (isopropyl or methyl substituents) of 2-isopropyl-5-methylcyclohexanol are oxidized [Yamaguchi *et al.*, 1994].

The biliary route of metabolism of 2-isopropyl-5-methylcyclohexanol appears to be more important in rodents and dogs than in humans and rabbits. *l*-2-Isopropyl-5-methylcyclohexanone given to rabbits (1000 mg/kg bw) [Williams, 1938, 1940] is stereoselectively reduced to *d* stereoisomer of 2-isopropyl-5-methylcyclohexanol [Williams, 1940].

Urine samples collected over the course of four (4) days from rabbits given 1000 mg/kg bw of isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) *via* gavage showed several metabolites: the three major conjugated metabolites include 3,5,5-trimethyl-2-cyclohexen-1-ol (isophorol), formed by reduction of the ketone group and then conjugation with glucuronic acid, *cis*- and *trans*-3,5,5-trimethylcyclohexanol formed by hydrogenation of the endocyclic double bond, reduction of the ketone, and conjugation with glucuronic acid, and 5,5-dimethyl-1-cyclohexene-3-one-1-carboxylic acid formed by methyl group oxidation at an exocyclic allylic position [Truhaut *et al*., 1970; Dutertre-Catella *et al*, 1978].

The data clearly demonstrate that unsubstituted or alkyl-substituted cyclohexanones are readily reduced to the corresponding cyclohexanol derivatives in a variety of animal species over a wide range of dose levels. The cyclohexanol derivatives are then conjugated with glucuronic acid and excreted mainly in the urine.

# 3 TEST PLAN

## 3.1 CHEMICAL AND PHYSICAL PROPERTIES

### 3.1.1 Melting Point

The melting point of 4-*tert*-butylcyclohexanol has been reported to be 56.6-58.6 ºC [Krestinina *et al*., 1984], 56-58 °C [General Aniline and Film Corp., 1965] and 55-70 ºC [Fragrance Materials Association (FMA), unpublished report], and has been calculated to be 4.34 °C [MPBPVPWIN EPI Suite, 2000].

The melting point of 4-*tert*-butylcyclohexyl acetate has been reported to be less than or equal to -50 ºC [Degussa AG, 2003b] and has been calculated to be 10.93 C [MPBPVPWIN EPI Suite, 2000].

The melting point of the isomeric alkyl-substituted cyclohexanol, 2-isopropyl-5-methylcyclohexanol was reported to be 41-43 ºC [Merck Index, 1997], 30 ºC (synthetic menthol) and 41 ºC (natural menthol) [Fragrance Materials Association (FMA), unpublished report].

Based on the above data, the melting points of 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are 56.6-58.6 ºC and less than or equal to -50 ºC, respectively.

### 3.1.2 Boiling Point

The boiling point of 4-*tert*-butylcyclohexanol has been reported to be 223-228 ºC at 1013 hPa [Degussa AG, 2003a, 1998a] and 110 ºC at 15 mm Hg [Fragrance Materials Association (FMA), unpublished report], and has been calculated to be 216.91 ºC [MPBPVPWIN EPI Suite, 2000].

The boiling point of 4-*tert*-butylcyclohexyl acetate has been reported to be approximately 241 ºC at 1013 hPa [Degussa AG, 2003b, 1998b] and 260 ºC [Fragrance Materials

Association (FMA), unpublished report], and has been calculated to be 232.55 ºC [MPBPVPWIN EPI Suite, 2000].

The boiling point of isomeric cyclohexanol 2-isopropyl-5-methylcyclohexanol was reported to be 212 ºC [Merck Index, 1997] and 216 ºC [Fragrance Materials Association (FMA), unpublished report]. Based on the measured boiling points values from a number of sources, the boiling points of 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are 224-228 ºC and 241 ºC, respectively.

### 3.1.3 Vapor Pressure

The vapor pressure of 4-*tert*-butylcyclohexanol has been reported to be less than 0.1 kPa or less than 0.75 mm Hg at 20 ºC [Degussa AG, 2003a]. The calculated vapor pressure has been estimated to be 0.005 mm Hg at 20 ºC [Fragrance Materials Association (FMA), unpublished report]. The calculated vapor pressure for 4-*tert*-butylcyclohexanol according to the MPBPVPWIN program is 0.0263 mm Hg at 25 °C.

The vapor pressure of 4-*tert*-butylcyclohexyl acetate has been reported to be 0.01 hPa or less than 0.075 mm at 20 ºC [Degussa AG, 2003b] and has been calculated to be approximately 0.067 kPa or less than 0.050 mm Hg at 20 ºC [Huels AG, 1985] and 0.03 mm Hg at 20 ºC [Fragrance Materials Association (FMA), unpublished report].

The calculated vapor pressure for 4-*tert*-butylcyclohexyl acetate according to the MPBPVPWIN program is 0.002 kPa or 0.0159 mm Hg at 25 °C.

The vapor pressure of isomeric cyclohexanol, 2-isopropyl-5-methylcyclohexanol, was reported to be 0.02 mm Hg at 20 ºC [Fragrance Materials Association (FMA), unpublished report]. Based on the experimental and calculated data, the vapor pressure of 4-*tert*-butylcyclohexanol is less than 0.1 kPa or less than 0.75 mm Hg at 20 ºC and vapor pressure of 4-*tert*-butylcyclohexyl acetate is 0.01 kPa or less than 0.075 mm Hg at 20 ºC

### 3.1.4 n-Octanol/Water Partition Coefficients

Log $K_{OW}$ for 4-*tert*-butylcyclohexanol calculated by different models, resulted in values of 3.42 [KOWWIN EPI Suite, 2000]. The calculated value exhibits good agreement with the measured log $K_{OW}$ value 3.23 [Degussa AG, 1981]. The measured value for 4-*tert*-butylcyclohexanol is in good agreement with the calculated log $K_{OW}$ value of 3.38 for the isomeric cyclohexanol derivative, 2-isopropyl-5-methylcyclohexanol [KOWWIN EPI Suite, 2000].

For 4-*tert*-butylcyclohexyl acetate, the calculated log $K_{OW}$ value of 4.42 [KOWWIN EPI Suite, 2000] is slightly less than the measured value of 4.8 [Givaudan-Roure, 1996].

Based on these data the log $K_{OW}$ values for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are 3.23 and 4.8, respectively.

### 3.1.5 Water Solubility

Reported experimental values for water solubilities for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are less than 100 [Degussa AG, 2003a] and approximately 90 mg/L [Degussa AG, 2003b] at 20 ºC, respectively while calculated values are determined to be 528.9 and 2.552 mg/L, respectively, at 25 $^{o}$C [WSKOWIN EPI Suite, 2000]. Based primarily on reported experimental values, the water solubility of the alcohol is concluded to be less than 100 mg/L at 20 ºC and water solubility of the acetate is concluded to be 90 mg/L at 20 ºC.

### 3.1.6 New Testing Required

No further testing is required.

## 3.2 ENVIRONMENTAL FATE AND PATHWAYS

### 3.2.1 Photodegradation

The calculated half-life values for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate have been reported to be 6.361 and 8.850 hours, respectively [AOPWIN EPI Suite, 2000]. The calculations are based on measured rate constants for radical reactions of OH, $O_3$ and $NO_3$ with organic substrates [AOPWIN EPI Suite, 2000]. The short half-life for the alcohol is consistent with the presence of reactive alcoholic OH function. Therefore, the half-life can be considered reliable.

### 3.2.2 Stability in Water

The calculated hydrolysis half-life for 4-*tert*-butylcyclohexyl acetate is 266 days at pH 8 and 7.2 years at pH 7 [HYDROWIN EPI Suite, 2000]. Other cyclohexanol esters are readily hydrolyzed *in vivo* (see Hydrolysis Section 2.4.1.)

### 3.2.3 Biodegradation

In a study adhering to OECD Guidelines, 4-*tert*-butylcyclohexanol was readily biodegradable (90% in 19 days) when tested using predominantly domestic sewage [Degussa AG, 1983].

4-*tert*-Butylcyclohexyl acetate was reported to be readily biodegradable (*i.e.*, >60% biodegradation within 10-day window) when tested with domestic activated sludge in the ISO BOD test for insoluble substances (68% in 28 days) [Degussa AG, 1995] and an aerobic evolution test (75% in 28 days) [Degussa AG, 1997b].

In the Manometric Respirometric test, 4-*tert*-butylcyclohexyl acetate was not readily biodegradable (54% after 28 days) [Rudio, 1996a], and was not inherently but partially

biodegradable (24% after 28 days) when determined by the Respirometric Method (modified MITI Test II) [Rudio, 1996b].

Given the database of information, 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are readily biodegradable.

### 3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1991, 1996a, 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, melting point and boiling point.

The model predicts that 4-*tert*-butylcyclohexanol is distributed mainly to the soil (59.7%), but also is distributed to water (37.9%) and, to a small extent, air (1.92%) and sediment (0.468%). In addition, the model predicts that 4-*tert*-butylcyclohexyl acetate is distributed mainly to the soil (71.3%), but also is distributed to water (14.9%) and sediment (12.1%) and, to a lesser extent, air (1.66%).

In these environmental compartments, released 4-*tert*-butylcyclohexanol exhibits a potential to be oxidized to the corresponding ketone while 4-*tert*-butylcyclohexyl acetate is appreciably hydrolyzed to the alcohol that is then oxidized to the ketone. Because of their use in cosmetics, soaps and detergents, the majority of 4-*tert*-butylcyclohexanol will enter the environment primarily *via* a sewage treatment plant and will be rapidly and extensively biodegraded. Therefore, low levels of the alcohol and ester will reach the environment.

### 3.2.5 New Testing Required

No further testing is required.

## 3.3 ECOTOXICITY

### 3.3.1 Acute Toxicity to Fish

Experimental and calculated acute toxicity data for fish were available for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate. In fresh water fish, the 48-hour static LC50 for 4-*tert*-butylcyclohexanol was reported to be 17 mg/L [Degussa AG, 1987]. Similarly, for 4-*tert*-butylcyclohexyl acetate, the 48-hour static LC50 was reported to be 14 mg/L [Degussa AG, 1985a] and a 96-hour semi-static LC50 was reported to be 8.6 mg/L [Degussa AG, 1997c].

For 4-*tert*-butylcyclohexanol, the calculated 96-hour LC50 was reported to be 8.085 mg/L (neutral organics) and the 14-day LC50 was calculated to be 17.805 mg/L [ECOSAR EPI Suite, 2000]. For 4-*tert*-butylcyclohexyl acetate, the calculated 96-hour LC50 was reported to be 0.954 mg/L (esters) [ECOSAR EPI Suite, 2000].

Given the consistency of measured and calculated data, it will not be necessary to perform additional acute fish toxicity tests. The 48-hour static LC50 for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate are 14-17 mg/L. The 96-hour semi-static LC50 is expected to be approximately 10 mg/L for 4-*tert*-butylcyclohexyl acetate.

### 3.3.2 Acute Toxicity to Invertebrates

Measured and calculated aquatic invertebrate LC50 values were available for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate. In *Daphnia magna*, the 48-hour EC50 values were determined to be 46 and 23.4 mg/L for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate, respectively [Degussa AG, 1994b, 1997a]. In two other studies, the 24-hour EC50 values for 4-*tert*-butylcyclohexyl acetate was determined to be 7.0 mg/L [Degussa AG, 1985b] and 19 mg/L [Degussa AG, 1985c].

In *Daphnia magna*, the calculated 48-hour LC50 values for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate were reported to be 9.431 mg/L and 0.446 mg/L, respectively [ECOSAR EPI Suite, 2000]. In mysid shrimp, a 96-hour LC50 value of 0.969 mg/L was calculated for 4-tert-butylcyclohexanol [ECOSAR EPI Suite, 2000].

### 3.3.3 Acute Toxicity to Aquatic Plants

Experimental and calculated acute toxicity data for aquatic plants were available for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate. 4-*tert*-Butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate were tested in *Scenedesmus subspicatus* (algae) and, based on growth rates, the 72-hour EC50 values of 45 and 17 mg/L, respectively, were determined [Degussa AG, 1992, 1994a].

Calculated 96-hour EC50 values of 6.329 mg/L for 4-*tert*-butylcyclohexanol and 0.084 mg/L for 4-*tert*-butylcyclohexyl acetate are at least an order of magnitude less than the experimentally determined values for green algae. This reflects the conservative nature of the model predictions [ECOSAR EPI Suite, 2000].

### 3.3.4 New Testing Required

No further testing is required.

## 3.4 HUMAN HEALTH

### 3.4.1 Acute Toxicity

Numerous oral, dermal, and intraperitoneal LD50 values for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate have been reported in rats, mice, and rabbits and have demonstrated the overall low acute toxic potential of chemicals in this category. Oral and dermal LD50s tended to exceed 4,000 mg/kg bw, whereas intraperitoneal LD50s in mice were much lower (less than 400 mg/kg bw).

For 4-*tert*-butylcyclohexanol, the rat oral LD50 was reported to be 4,200 mg/kg bw [Denine and Palanker, 1973]. For 4-*tert*-butylcyclohexyl acetate, the rat oral LD50 values were reported to range from greater than 500 to 5,000 mg/kg bw [Zeller and Hofmann, 1970; Moreno, 1976; Opdyke, 1976]. Similar values have been reported for 2-isopropyl-5-methylcyclohexanol (940 - 4,384 mg/kg bw) [Jenner *et al*., 1964; Food and Drug Administration (FDA), 1975].

Dermal LD50s in rabbits were reported to be greater than 5,000 mg/kg bw for both 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate [Denine and Palanker, 1973; Opdyke, 1976].

The mouse intraperitoneal LD50 for 4-*tert*-butylcyclohexanol was 50-100 mg/kg bw [Doull *et al*., 1962] and for 4-*tert*-butylcyclohexyl acetate was 400 ml/kg bw [Zeller and Hofmann, 1970].

Given the current database of information, it will not be necessary to perform additional acute toxicity tests.

### 3.4.2 *In vitro* and *In vivo* Genotoxicity

Both chemicals in this category have been tested in *in vitro* bacterial and mammalian studies and have shown no mutagenic or genotoxic potential. Similar results have been reported for the isomeric cyclohexanol derivative, 2-isopropyl-5-methylcyclohexanol, for which a more extensive database was available. Although no *in vivo* genotoxicity data exist for 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate, studies are available for 2-isopropyl-5-methylcyclohexanol and the results confirm the findings of the *in vitro* studies that the alkyl-substituted cyclohexanol derivatives exhibit a low genotoxic potential.

#### *3.4.2.1 In vitro Genotoxicity*

4-*tert*-Butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate have shown no mutagenic potential when tested at concentrations up to 5,000 micrograms/plate in the Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 with or without metabolic activation [Degussa AG, 1989, 1988a]. 2-Isopropyl-5-methylcyclohexanol was not mutagenic when tested in *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, or TA98 with metabolic activation at concentrations up to 500 micrograms/plate [Ishidate *et al*., 1984] and when tested in *Salmonella typhimurium* strains TA100, TA2637 or TA98 with or without metabolic activation at concentrations up to 200 micrograms/plate [Nohmi *et al*., 1985].

The possible clastogenicity of 4-*tert*-butylcyclohexanol was studied in Chinese hamster V79 cells in the presence and absence of S9 [Degussa AG, 1997]. No biologically significant increases in chromosomal aberrations were reported and 4-*tert*-butylcyclohexanol was considered by the authors to be non-clastogenic in this experiment.

2-Isopropyl-5-methylcyclohexanol did not induce an increase in the incidence of chromosomal aberrations or an increased frequency of sister chromatid exchanges (SCEs)

in human lymphocytes at concentrations up to 10 mM, with or without metabolic activation [Murthy *et al*., 1991], and also did not induce chromosomal aberrations in Chinese hamster fibroblasts when tested at concentrations up to 200 micrograms/ml [Ishidate *et al*., 1984].

As part of the National Toxicology Program (NTP), 2-isopropyl-5-methylcyclohexanol was not mutagenic when tested in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 with or without metabolic activation at concentrations up to 666 micrograms/plate [Zeiger *et al*., 1988], tested negative for chromosomal aberrations and SCEs in Chinese hamster ovary cells [Ivett *et al*., 1989], and did not increase mutation frequency in mouse lymphoma cells at concentrations up to 150 micrograms/ml or even at cytotoxic concentrations of 200 micrograms/ml [Myhr and Caspary, 1991].

2-Isopropyl-5-methylcyclohexanol was not clastogenic in human embryonic lung cultures when tested at concentrations up to 10 micrograms/ml *in vitro* [Food and Drug Administration (FDA), 1975].

#### *3.4.2.2 In vivo Genotoxicity*

Intraperitoneal injection on 3 consecutive days with up to 1,000 mg 2-isopropyl-5-methylcyclohexanol/kg bw did not induce micronuclei in mouse bone marrow [Shelby *et al*., 1993].

In a host-mediated assay, mice were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol in a single-dose study or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol in a 5-day study [Food and Drug Administration (FDA), 1975]. After the last dose, mice were intraperitoneally injected with an indicator organism (*Salmonella typhimurium* strains G46 and TA1530, or *Saccharomyces cervisiae* D3). The peritoneal exudate was plated and incubated for assessment of mutation and recombinant frequencies. No significant increase in mutant and recombinant frequency was observed at any dose or exposure period in *Salmonella typhimurium* G46. In

*Saccharomyces cervisiae* D3, an elevation of recombinant frequency was reported in the 5-day exposure study, but not in the single-exposure study. At the highest dose tested in *Salmonella typhimurium* TA1530 in the single-dose study, a significant increase in mutant frequency was reported. This was not reported in the 5-day study. *In vitro* tests using the same organisms were all negative.

In a chromosomal aberration study, rats were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol as a single exposure or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol for 5 days [Food and Drug Administration (FDA), 1975]. Analysis of bone marrow demonstrated that exposure to 2-isopropyl-5-methylcyclohexanol as a single dose or for a 5-day period did not induce chromosomal aberrations.

#### *3.4.2.3 Conclusions*

The *in vitro* studies on 4-*tert*-butylcyclohexanol and 4-*tert*-butylcyclohexyl acetate indicate that these substances exhibit no mutagenic or genotoxic potential. The isomeric cyclohexanol derivative, 2-isopropyl-5-methylcyclohexanol, with a larger safety database, also shows no genotoxic potential *in vitro* and these results are supported by findings in whole animals. Therefore, it is concluded that no additional genotoxicity studies are required for this chemical category.

### 3.4.3 Repeat Dose Toxicity

Repeat-dose toxicity studies are available for 4-*tert*-butylcyclohexanol and a mixture predominantly composed of isomeric alkyl-substituted cyclohexanol and cyclohexanone derivatives.

### *3.4.3.1 28-day Study for 4-tert-Butylcyclohexanol*

Groups of rats were gavaged with 0, 50, 150, or 300 mg/kg bw/day of 4-*tert*-butylcyclohexanol for 28 days with a 14-day post-observation period [Degussa AG, 1999]. Clinical signs, body weight, food and water consumption were monitored during the study and after 28 days, behavioral tests were conducted and rats were killed and necropsied. Two high-dose rats died due to gavage error and were replaced. Clinical signs at the 2 highest doses included convulsions, squatting position, straub tail and vocalization. The signs disappeared within a few hours to 1 day. No clinical abnormalities were observed in the controls and rats in the 14-day observation period. After 2, 3 and 4 weeks of treatment, behavioral observations in individual rats (predominantly from the high-dose group) were made and included ataxia, fasciculations, padding movements, defense against touching, aggressiveness, hunchback/squatting position, reduced respiration, hyperactivity, straub tail, and slight convulsions. In the recovery period (week 5 and 6), no significant treatment-related clinical signs were observed in any treatment group. High-dose recovery group males showed a statistically significant increase of group mean values of landing-foot-splay and rearing and a decrease of group mean values of grip strength compared to recovery controls, but these differences were minor and did not show a consistent pattern in the individual animals. The effects were also not seen in the high-dose group males that were not allocated to the recovery group at the same examination time. Therefore the findings were considered of minor toxicological importance. No statistically significant increase in motor activity was observed in any dose group. A slight reduction in food consumption was seen in treated males, whereas, females showed an increase in food consumption when compared to controls. During the recovery period the food consumption of the treated animals was increased compared to controls. Water consumption was not different between treated and control groups. Alterations in clinical chemistry, urinalysis and hematology parameters were minor and within the normal range of the historical data. All differences observed were considered of minor toxicological importance. At the end of the treatment period high-dose male rats showed a statistically significant increase in relative adrenal weight when compared with controls. At the end of the recovery period, treated males

exhibited a statistically significant increase in relative epididymis weight compared to the controls. However, there was no evidence of histopathology of this tissue. There were no other histopathological findings related to the reproductive organs of males (testes) or females (ovaries). Treatment-related histopathological findings were restricted to an increased number of male animals of the high-dose group with eosinophilic hyaline droplets in the epithelial cell cytoplasm of the proximal tubules (5 treated compared to 1 control). The authors proposed that the effect may be indicative of the *alpha*-2 micro-globulin nephropathy syndrome that is a rat-specific effect (see data and information included in study in section 3.4.3.2). Based on clinical signs, a no observable adverse effect level (NOAEL) of 50 mg/kg bw/day and a lowest observable adverse effect level (LOAEL) of 150 mg/kg bw/day were derived.

#### *3.4.3.2 28-Day Study with Mixture of Alkyl-substituted Cyclohexanol and Related Ester Derivatives*

A sample of an essential oil, predominantly containing a mixture of 2-isopropyl-5-methylcyclohexanol and 2-isopropyl-5-methylcyclohexanone isomers that accounts for greater than 85% of the mass of the oil, was used in the 28 day study [Serota, 1990] and reproductive/developmental screening [Hoberman, 1989] study cited below. Based on a gas chromatogram (FIS detector), the oil was determined to contain:

- 46.8% (1 *alpha*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol
- 3.97% (1 *alpha*, 2 *alpha*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol
- 0.86% (1 *beta*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanol
- 21.81% (2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexanone
- 3.07% (2 *beta*, 5 *beta*)-2-isopropyl-5-methylcyclohexanone
- 5.11% (1 *alpha*, 2 *beta*, 5 *alpha*)-2-isopropyl-5-methylcyclohexyl acetate
- 1.55% (1 *beta*, 2 *beta*, 5 *beta*)-2-isopropyl-5-methylcyclohexyl acetate

The other constituents accounting for approximately 10% of the oil included aliphatic terpene hydrocarbons (*e.g.*, *alpha*-pinene) and ethers (eucalyptol) (see Vollmuth, 1989 in Serota, 1990 reference).

The sample was administered by gavage in corn oil to groups of Sprague-Dawley rats at dose levels of 0, 100, 200, or 400 mg/kg bw/day for 29 or 30 days [Serota, 1990]. Clinical signs, body weights and food consumption were monitored. At necropsy, organ weights (brain, spleen, liver, heart, kidneys, testes with epididymides, adrenals, ovaries, and pituitary) were measured, and tissues (26) were preserved in 10% formalin. All tissues from the control and high-dose groups and tissues from the heart, liver, kidneys, and gross lesions from the low- and mid-dose group were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically. All animals survived to study termination with high-dose males showing increased incidence of urine staining during clinical observations. Except for a non-statistically significant decrease in mean body weight in high-dose males, there were no statistically significant differences in body weight or food consumption between treated and control groups. A significant decrease in serum glucose levels was reported in the mid- and high-dose males that the authors, in part, attribute to change in nutritional status as revealed by decreased body weights in the high-dose group. A treatment-related increase in alkaline phosphatase also was reported in high-dose males. Measurement of body weight, food consumption, hematology and clinical chemistry parameters revealed no significant changes between test and control female rats. There were statistically significant increases in relative kidney weights in high-dose males. Histopathological findings revealed renal tubule protein droplets in all groups of treated male rats. The authors considered these findings related to the lysosomal handling of *alpha*-2 micro-globulin, a protein specific to the male Sprague-Dawley rat. Absolute and relative liver weights in high-dose females also were significantly increased but these changes were not confirmed by histopathological examination. There was no histopathology of tissues from reproductive organs of males (testes with epididymis) or female (ovaries). Based exclusively on the renal pathology reported in all dosed groups of male rats, the authors concluded that the NOAEL for the sample is less than 100 mg/kg bw/day in male rats and 400 mg/kg bw/day in female rats.

### *3.4.3.3 Interpretation of alpha-2 Micro-globulin Data*

The mechanism of *alpha*-2-micro-globulin formation in the male rat has been the subject of intensive research for the last decade. Because this mechanism of action is widely applicable to a broad range of compounds using different modes of administration, no robust summaries have been prepared for the research results described below.

Since publication of the reports on the 28-day studies on 4-*tert*-butylcyclohexanol [Degussa AG, 1999] and an essential oil containing a mixture of 2-isopropyl-5-methylcyclohexanol and 2-isopropyl-5-methylcyclohexanone isomers 2-isopropyl-5-methylcyclohexanol [Serota, 1990], the mechanism of action associated with the formation of *alpha*-2 micro-globulin in male rats has been extensively studied. It has been clearly demonstrated that renal lesions, which were also observed in numerous NTP studies, resulted from the accumulation of aggregates of *alpha*-2 micro-globulin (a low molecular-weight protein synthesized in the liver) and test agents or their metabolites in the P2 segment of the renal proximal tubule. This phenomenon was initially observed in the male F344/N rat (Strasser *et al.*, 1988; Borghoff *et al.*, 1990) but has now been identified in other well-recognized strains of laboratory rats (Hildebrand *et al.*, 1997; Saito *et al.*, 1996).

The gene that encodes *alpha*-2micro-globulin has been isolated and the sequence deduced (Untermann *et al.*, 1981). These proteins are expressed in the liver under hormonal control (Roy and Neuhaus, 1967; Wang and Hodgetts, 1998). *alpha*-2 Micro-globulin belongs to the *alpha*-2 micro-globulin super family of proteins that are characterized by a unique hydrophobic binding pocket. The lesions do not develop in the female rat or in humans (Bucher *et al.*, 1986). Subsequent investigations have shown that the *alpha*-2 micro-globulin nephropathy found in the male rat does not develop in mammals that do not express the hepatic form of *alpha*-2 micro-globulin (Swenberg *et al.*, 1989; Dietrich and Swenberg, 1991), mice (Bucher *et al.*, 1986; Lehman-McKeeman and Caudill, 1994) and dogs (Webb *et al.*, 1990).

Transgenic mice that express rat *alpha*-2 micro-globulin were tested for their ability to form hyaline droplets and develop nephropathies similar to their adult male rat counterparts (Lehman-McKeeman and Caudill, 1994). This study involved male rats as positive control, transgenic C57BL/6J mice as experimental group and native C57BL/6 mice as negative controls. The animals at age 70-75 days were placed in metabolic cages and received 150 mg/kg bw/day of *d*-limonene in corn oil by gavage for three days. Limonene is a potent inducer of renal nephropathy in adult male rats (Environmental Protection Agency, 1991; National Toxicology Program, 1990). Twenty-four (24) hours after the last dose the animals were sacrificed and the kidneys analyzed for evidence of nephropathy. Hyaline droplet formation was evaluated on a subjective scale, size and intensity (0-4) multiplied by tubular loading (0-3) for an overall scale of 0-12 with 12 being the most severe. In the absence of *d*-limonene the control groups transgenic mice and rats showed a hyaline droplet score of 1+/-0 and 6+/-0.5, respectively. The test transgenic mice and rats showed a hyaline droplet score of 2.5+/-0.3 and 11+/-1.3, respectively upon dosing with *d*-limonene. The native mice developed no signs of hyaline droplet formation and tested negative for presence of *alpha*-2 micro-globulin in their urine. The authors assert that based on the data presented "*alpha*-2 micro-globulin is the only protein that is involved in the etiology of hyaline droplet nephropathy".

An increase in the kidney-type-*alpha*-2 micro-globulin was seen in male Sprague-Dawley rats when these animals were administered 200 mg/kg bw/day of isophorone by gavage for 7 days. The increases in the urinary kidney-type-*alpha*-2 micro-globulin are dose-dependent and parallel-elevated accumulation in the kidney cells (Saito *et al.*, 1996).

In another study, adult male Wistar rats were administered two groups of chemical compounds, including 138 mg/kg bw of isophorone, potassium bromate, 2-propanol and a series of benzene and anthracene derivatives, to study induction of accumulation of *alpha*-2 micro-globulin and structure-activity relationships. A monoclonal antibody against *alpha*-2 micro-globulin was employed in a competitive ELISA procedure to determine its concentration in urine or tissue samples without purification. Plasma concentrations of *alpha*-2 micro-globulin were not significantly increased by any of the

test compounds at 1 mmol/kg bw. Kidney tissue concentrations were found to be 297-300% higher than that of controls. The hyaline droplet accumulating (HDA) potential was dependent on the test compound but there was no relationship between HDA activity and the structure or the pathway used to metabolize the test substance (Hildebrand *et al*., 1997).

The above studies depend exclusively on histopathologic evidence to detect *alpha*-2 micro-globulin nephropathy. An *in vitro* assay based on the prerequisite that a chemical or metabolite bind to *alpha*-2 micro-globulin has been developed. The assay predicts, in greater than 90% (22/24) of the substances tested, the ability to induce *alpha*-2 micro-globulin nephropathy (Lehman-McKeeman and Caudill, 1999). *d*-Limonene-1,2-epoxide is well characterized as an *alpha*-2 micro-globulin nephropathy inducer and has a steady state binding constant ($K_d$) of 5 x $10^{-7}$ M (Lehman-McKeeman *et al.*, 1989). Based on this, a competitive binding assay was developed with [$^{14}$C]-d-limonene-1,2-epoxide and male rat urinary protein concentrate. Homogenous *alpha*-2 micro-globulin was obtained from adult male rats (Lehmann-McKeeman and Caudill, 1992). The assay was run with three series of competitive inhibitors terpenes (5), decalin/decanes (10), and halobenzenes (8). Total male urinary protein was incubated for 1 hour with the test materials, ranging from 0.001 to 3000 microM, and 0.5 microM [$^{14}$C]-d-limonene-1,2-epoxide. The ability of the test materials to displace 50% of the radiolabelled limonene epoxide from the protein was evaluated and IC50 values were calculated. An IC50 value of less than or equal to 100 microM for the terpene and decalin/decanone series is considered predictive of *alpha*-2 micro-globulin droplet formation. Substances with an IC50 calculated at higher than 100 microM in the competitive binding assay were subjected to microsomal oxidation to generate metabolites that would bind to *alpha*-2 micro-globulin. Three of the halobenzenes 1,2-, 1,4-, and 1,3-dichlorobenzene tested positive for *alpha*-2 micro-globulin binding when incubated in the presence of rat liver microsomes. Parallel *in vivo* tests were performed in rats and hyaline droplet formation in the kidney was assessed to confirm the *in vitro* results. The authors concluded that the *in vitro* assay is greater than 90% predictive of *alpha*-2 micro-globulin nephropathy induction in male rats without

being invasive or requiring additional animal testing (Lehman-McKeeman and Caudill, 1999).

To further investigate kidney tissue concentration of *alpha*-2 micro-globulin in the lysosomal portion, intact kidney lysosomes were isolated from untreated or 2,2,4-trimethylpentane (TMP)-treated rats and their ability to take up *alpha*-2 micro-globulin was compared. It was found that *alpha*-2 micro-globulin could be directly taken up in the presence of the heat shock cognate protein (*hsc*73). Hsc73 contributes to the normal degradation, lysis, of *alpha*-2 micro-globulin in rat kidney and liver. However, in the presence of a chemical (TMP) known to induce aggregation of *alpha*-2 micro-globulin, the activity of this pathway is increased. This may be due to an increase in the concentration of a receptor protein in the lysosomal membrane, which accelerates the uptake of the cytosolic protein, *alpha*-2 micro-globulin (Cuervo *et al.*, 1999).

While humans produce low molecular weight serum proteins, which are reabsorbed by the kidney, there is no evidence that *alpha*-2 micro-globulin is produced (Olson *et al.*, 1990). Urine collected from adult male rats and humans revealed no evidence that *alpha*-2 micro-globulin production occurs in humans (Olson *et al.*, 1990).

It is unknown whether any human serum proteins possess a binding site similar to that of *alpha*-2 micro-globulin. Although this is a possibility, it appears remote, since female rats, mice, and dogs do not show the renal changes noted in male rats exposed to isophorone. It should be noted that there is a class of human proteins referred to as the *alpha*-2 micro-globulin related proteins. They appear to have no functional relationship to the adult male rat urine proteins. The human protein has a higher molecular weight, 25 kDa and is a component of a neutrophil gelatinase complex (Kjeldsen *et al.*, 2000; Triebel *et al*., 1992). An extensive review of the current scientific literature and genome databases reveals no native protein or biological entity that acts as a nephropathic agent like mature male rat *alpha*-2 micro-globulin. The accumulated evidence indicates that it is the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows isophorone to interfere with renal processing of the strain-specific *alpha*-2 micro-globulin. Therefore, this

process is not predictive of human carcinogenicity. In a comprehensive review of *alpha*-2 micro-globulin nephropathy and associated renal tubule tumors produced in the male rat exposed to isophorone and other simple chemical substances (*e.g.*, limonene, decalin and methyl isobutyl ketone), it was concluded that the F344/N male rat is not an appropriate model for assessing human renal carcinogenic risk (Environmental Protection Agency, 1991). After careful review, it has been concluded that the mechanisms leading to the renal carcinogenic findings in the male rat are largely known and strongly indicate that the nephropathy associated with male rats have no significance for human risk assessment (Burdock *et al*., 1990).

Based on the results of these studies, it can be concluded that the renal pathology reported in male rats treated with 4-*tert*-butylcyclohexanol or the mixture containing greater than 85% of alkyl substituted cyclohexanol derivatives is unrelated to the human health assessment. Therefore, with the exception of the renal effects reported in male rats, the NOAEL for male or female rats given the mixture of 2-isopropyl-5-methylcyclohexanol is 400 mg/kg bw/day and the NOAEL for male and female rats given 4-*tert*-butylcyclohexanol is 50 mg/kg bw/day.

#### *3.4.3.4 Chronic Studies*

##### ***3.4.3.4.1 Mice***

B6C3F1 mice were fed diets containing 0, 930, 1870, 3750, 7500, or 15,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 140, 281, 563, 1125 or 2,250 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol, respectively) for 13 weeks [National Cancer Institute, 1979]. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from selected animals. Six mice (sex not specified) died during the study but the deaths could not be attributed to compound administration. Final mean body weights of the male mice and female mice were not statistically different from those of the controls except for the high-dose female group which showed statistically significant decreased body weights. A slight increase in

the incidence of perivascular lymphoid hyperplasia and interstitial nephritis was reported in female mice given the two highest dose levels. No adverse effects were reported for male or female mice administered 140, 281, or 563 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol.

A carcinogenicity study was conducted in which groups of B6C3F1 mice of each sex were fed diets containing 0, 2,000 or 4,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 300, or 600 mg/kg bw/day, respectively) for 103 weeks [National Cancer Institute, 1979]. Necropsies and histological examinations were performed on all animals at the termination of the study and on those found dead during the study. The mean body weights of the treated mice were slightly lower than those of controls. Survival of the treated male mice and low-dose female mice was similar to the vehicle control animals; however, survival of the high-dose group of female mice was significantly less than that of the control animals but was not accompanied by any evidence of toxicity. There was no evidence of neoplastic or nonneoplastic lesions of the male (penis, prepuce, preputial gland, prostate, or epididymis) or female (uterus, endometrium, or ovaries) reproductive system. An increase in the incidence of hepatocellular carcinomas was observed in high-dose male mice, but was not statistically different from that observed historically in control mice of that age and strain (Haseman *et al*., 1986, no robust summary provided). A low incidence of alveolar/bronchiolar adenomas of the lung was observed in treated females but was not statistically different from the incidence of this neoplasm in historical control groups. Under the conditions of this study, the authors concluded that *dl*-2-isopropyl-5-methylcyclohexanol was not carcinogenic and did not produce any organ-specific toxicity for either sex of B6C3F1 mice at dose levels up to 600 mg/kg bw/day.

#### *3.4.3.4.2 Rats*

Fischer 344 rats were fed diets containing 0, 930, 1870, 3750, 7500, or 15,000 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 93, 187, 375, 750 or 1500 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol, respectively) for 13 weeks [National

Cancer Institute, 1979]. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from selected animals. Final mean body weights of the male and female rats at all dose levels were similar to those of the controls. A slight increase in the incidence of interstitial nephritis was observed in high-dose male rats. This effect may have been related to the presence of *alpha*-2 micro-globulin, but at time of the study (*i.e.*, 1979) the *alpha*-2 micro-globulin phenomenon in the male rat kidney had yet been characterized. No adverse effects were reported for male or female rats administered up to 750 mg/kg bw/day of *dl*-2-isopropyl-5-methylcyclohexanol.

Fischer 344 rats of each sex were fed diets containing 0, 3,750, or 7,500 ppm *dl*-2-isopropyl-5-methylcyclohexanol (approximately 0, 187, or 375 mg/kgbw/day of *dl*-2-isopropyl-5-methylcyclohexanol, respectively) for 103 weeks [National Cancer Institute, 1979]. Necropsies and histological examinations were performed on all animals at the termination of the study and on those found dead during the study. The mean body weights treated rats were slightly lower when compared to the controls. Microscopic examination of tissues of test animals failed to reveal any evidence of neoplastic or nonneoplastic lesions, including those of the male (*e.g.*, penis, scrotum, prostate, mammary gland, or epididymis) or female (uterus, vagina, mammary gland, endometrium, or ovaries) reproductive system. Survival of the treated rats was similar to the control animals. Chronic inflammation of the kidney observed in the dosed older males was not considered by the authors to be related to the administration of *dl*-2-isopropyl-5-methylcyclohexanol since the effect is commonly observed in aged male Fischer 344 rats. There was no increase in the incidence of neoplasms of dosed females compared to that of control animals. In treated females, fibroadenomas of the mammary glands occurred at a lower incidence than in the control group. Alveolar/bronchiolar adenomas or carcinomas were reported only for the female control rats. There was no report of *alpha*-2 micro-globulin-induced nephropathy of male rats. This is not unexpected given that this phenomenon was identified only in subsequent NTP sponsored bioassays. Under the conditions of this study, the authors concluded that *dl*-2-isopropyl-

5-methylcyclohexanol was neither carcinogenic nor toxic for either sex of Fischer 344 rats at dose levels of up to 375 mg/kg bw of *dl*-2-isopropyl-5-methylcyclohexanol.

The extensive database of repeat dose studies for 4-*tert*-butylcyclohexanol and the isomeric alkyl-substituted cyclohexanol derivative, 2-isopropyl-5-methylcyclohexanol indicate that these substances exhibit no carcinogenic potential and a very low order of subchronic and chronic toxicity. Therefore, it is not necessary to conduct additional studies on 4-*tert*-butylcyclohexanol or 4-*tert*-butylcyclohexyl acetate.

### 3.4.4 Reproductive Toxicity

Virgin Crl CD rats were administered oral dose levels of 0, 150, 750, or 1,500 mg/kg bw/day of the 2-isopropyl-5-methylcyclohexanol by gavage for 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Hoberman, 1989]. The composition of the test material was identical to that used in the previously reported 28-day study [Serota, 1990]. The study design included measurement of parameters for reproductive and developmental toxicity. Maternal indices monitored included twice-daily clinical observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, and number of offspring per litter). Offspring indices monitored included daily observation, clinical signs, examination for gross external malformations, and measurement of mortality (number of stillborns), viability (pups dying on days 1-4), body weight and body weight gain.

At the two highest dose levels, maternal mortality was increased; significant decreases in maternal body weight and food consumption were reported. Clinical observations of the dams included decreased motor activity, ataxia, dysnea, rales, chromorrhinorrhea, un-groomed coat and thin appearance, and significant increases in pup mortality. Live litters were reported for 9/19, 8/10, 5/6, and 1/4 pregnant females in the control, 150, 750, and 1,500 mg/kg bw/day groups, respectively. Increases in the numbers of dams with

stillborn pups, stillborn pups, and late resorptions *in utero* were reported only in the mid-dose group. At the highest dose, 2 rats had only resorptions *in utero* when found dead on gestation day 23 and one rat possessed only empty implantation sites *in utero* on day 25 of presumed gestation. Even at the highest dose level, there was no evidence of an effect of the test article on implantation, duration of gestation, pup sex ratio, or gross morphology of pups. Based on these results the authors concluded that the maternal NOAEL for reproductive effects was 150 mg/kg bw/day and the offspring NOAEL for developmental effects is greater than 150 mg/kg bw/day, but less than 750 mg/kg bw/day.

In a dominant lethal assay, males rats were gavaged with up to 3,000 mg/kg bw of 2-isopropyl-5-methylcyclohexanol as a single exposure or up to 1,150 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol for 5 days [Food and Drug Administration, 1975]. Male rats were mated with 2 female rats per week for 7-8 weeks following the last treatment. Fourteen days after mating, females were killed and the uteruses examined for early deaths, late fetal deaths, and total implantations. No effect on early deaths, late fetal deaths or total implantations was reported when 2-isopropyl-5-methylcyclohexanol was administered to male rats prior to mating.

Given the lack of significant reproductive effects in the reproductive/developmental screening study and the absence of any significant effects to the reproductive organs of animals in subchronic and chronic repeat-dose studies, it is concluded that alkyl-substituted cyclohexanol exhibits a very low order of reproductive toxicity.

### 3.4.5 Developmental Toxicity

Teratology studies in four animal species were performed under Food and Drug Administration contracts for the isomer of 4-*tert*-butylcyclohexanol, 2-isopropyl-5-methylcyclohexanol. Studies in mice [Morgareidge, 1973a], rats [Morgareidge, 1973b], and hamsters [Morgareidge, 1973c] were performed using the same study design. In each study, virgin adult females (CD-1 outbred mice, Wistar rats, or golden hamsters) were mated with untreated young adult males and observation of vaginal sperm plugs was

considered day 0 of gestation. Beginning on day 6 and continuing daily through day 15 (mice and rats) or day 10 (for hamsters) of gestation, groups (22-23 for mice, 22-25 for rats and 19-23 for hamsters) of pregnant females were given 2-isopropyl-5-methylcyclohexanol by gavage in corn oil. Mice received 0, 1.85, 8.59, 39.9, or 185 mg/kg bw/day, rats received 2.18, 10.15, 47.05, or 218 mg/kg bw/day, and hamsters received 0.05, 21.15, 98.2, or 405 mg/kg bw/day. Negative control groups received corn oil by gavage daily while positive control groups received aspirin. On day 17(mice), 20 (rats), or 14 (hamsters), all dams were subjected to Caesarian section and the number of live litters, implantation sites, number of resorptions, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, implant sites per dam, percent of live and percent partial live resorptions, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10X magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. No effects on any of the above-described parameters were reported in any of the species tested and the authors concluded that there was no evidence of maternal or developmental toxicity at dose levels up to and including 185 (mice), 218 (rats), and 405 (hamsters) mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol during gestation.

Virgin adult female rabbits were artificially inseminated and beginning on gestation day 6 and continuing daily through day 18, pregnant rabbits were given 0, 4.25, 19.75, 91.7, or 425 mg/kg bw of 2-isopropyl-5-methylcyclohexanol by gavage in corn oil [Morgareidge, 1973d]. A positive control group received 2.5 mg/kg bw/day of 6-aminonicotinamide. On gestation day 29 all dams were subjected to Caesarian section and the number of *corpora lutea*, implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were recorded. The urogenital tract of each dam was examined for anatomical abnormalities. All live fetuses were placed in an incubator for 24 hours and evaluated for survival. All surviving pups were sacrificed and subjected to detailed visceral examination at 10X magnification. All fetuses were cleared

with KOH, stained with alizarin red S dye, and examined for skeletal defects. As reported for the 3 other species, there was no evidence of either maternal toxicity or developmental toxicity at dose levels up to and including 425 mg/kg bw/day of 2-isopropyl-5-methylcyclohexanol. Given the results of this multiple species study, alkyl-substituted cyclohexanol derivatives exhibit a low potential for developmental toxicity.

### 3.4.6 New Testing Required

No further testing is required.

## 3.5 TEST PLAN TABLE

| Chemical | Physical-Chemical Properties | | | | |
|---|---|---|---|---|---|
| | **Melting Point** | **Boiling Point** | **Vapor Pressure** | **Partition Coefficient** | **Water Solubility** |
| **CAS NO. 98-52-2** 4-*tert*-butylcyclohexanol | A, Calc | A, Calc | A, Calc | A, Calc | A, Calc |
| **CAS NO. 32210-23-4** 4-*tert*-butylcyclohexyl acetate | A, Calc | A, Calc | A, Calc | A, Calc | A, Calc |

| Chemical | Environmental Fate and Pathways | | | |
|---|---|---|---|---|
| | **Photo-degradation** | **Stability in Water** | **Biodegradation** | **Fugacity** |
| **CAS NO. 98-52-2** 4-*tert*-butylcyclohexanol | Calc | NA | A | Calc |
| **CAS NO. 32210-23-4** 4-*tert*-butylcyclohexyl acetate | Calc | Calc | A | Calc |

| Chemical | Ecotoxicity | | |
|---|---|---|---|
| | **Acute Toxicity to Fish** | **Acute Toxicity to Aquatic Invertebrates** | **Acute Toxicity to Aquatic Plants** |
| **CAS NO. 98-52-2** 4-*tert*-butylcyclohexanol | A, Calc | A, Calc | A, Calc |
| **CAS NO. 32210-23-4** 4-*tert*-butylcyclohexyl acetate | A, Calc | A, Calc | A, Calc |

| Chemical | Human Health Data | | | | | |
|---|---|---|---|---|---|---|
| | **Acute Toxicity** | **Genetic Toxicity *In Vitro*** | **Genetic Toxicity *In Vivo*** | **Repeat Dose Toxicity** | **Repro-ductive Toxicity** | **Develop-mental Toxicity** |
| **CAS NO. 98-52-2** 4-*tert*-butylcyclohexanol | A | A | R | A | R | R |
| **CAS NO. 32210-23-4** 4-*tert*-butylcyclohexyl acetate | A | A | R | R | R | R |

| Legend | |
|---|---|
| **Symbol** | **Description** |
| R | Endpoint requirement fulfilled using category approach, SAR |
| Test | Endpoint requirements to be fulfilled with testing |
| Calc | Endpoint requirement fulfilled based on calculated data |
| A | Endpoint requirement fulfilled with adequate existing data |
| NR | Not required per the OECD SIDS guidance |
| NA | Not applicable due to physical/chemical properties |
| O | Other |

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# The Flavor And Fragrance High Production Volume Consortia

## The Cyclohexyl Derivatives Consortium

## Robust Summaries for Alkyl-substituted Cyclohexanol Derivatives

| | |
|---|---|
| **4-*tert*-butylcyclohexanol** | **CAS No. 98-52-2** |
| **4-*tert*-butylcyclohexyl acetate** | **CAS No. 32210-23-4** |

## FFHPVC Cyclohexyl Derivatives Consortium Registration Number

**Submitted to the EPA under the HPV Challenge Program by:**

**The Flavor and Fragrance High Production Volume Chemical Consortia**

**1620 I Street, NW, Suite 925**

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# List of Member Companies

**FIRMENICH, INC.**

**INTERNATIONAL FLAVORS & FRAGRANCES INC.**

**J. MANHEIMER, INC.**

**QUEST INTERNATIONAL**

**DEGUSSA AG/DEGUSSA CORPORATION**

# Table of Contents

# The Flavor and Fragrance High Production Volume Consortia

# Robust Summaries for Alkyl-substituted Cyclohexanol Derivatives

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

# 1 CHEMICAL AND PHYSICAL PROPERTIES

## 1.1 Melting Point

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Melting Point** | 56.6-58.6 °C |
| **Remarks for General Remarks** | Handbook data (Beilstein) |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Krestinina, T. B.; Koshel', G. N.; Chabutkina, E. M.; Antonova, T. N.; Migachev, G. I.; JAPUAW; J.Appl.Chem.USSR |

| | |
|---|---|
| | (Engl.Transl.); EN; 57; 9; 1984; 2138-2142; ZPKHAB; Zh.Prikl.Khim. (Leningrad); RU; 57; 9; 1984; 2318-2324; |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **GLP** | No |
| **Year** | 1965 |
| **Melting Point** | 56-58 °C |
| **Remarks for General Remarks** | Handbook data, Beilstein data base |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | General Aniline + Film Corp.; Patent; FR 1411988; 1965; Chem.Abstr.; EN; 64;1983c; 1966; |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | 55-70 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculated. Mean or weighted MP. |
| **Melting Point** | 4.34 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |

| | |
|---|---|
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Melting Point** | 41-43 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4.Only secondary literature (review, tables, books, etc.). |
| **References** | Merck Index (1997) Merck & Co., Inc. Whitehouse Station, NJ. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Melting Point** | 30 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Melting Point** | 41 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | ASTM-D-1015 |
| **GLP** | No |
| **Melting Point** | Less than -50 °C |

| | |
|---|---|
| **Decomposition** | No at C |
| **Sublimation** | No |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003b) Safety Data Sheet for 4-*tert*-Butylcyclohexyl acetate.<br>Degussa AG (1998) Product information, Data Sheet 1401, 1.12. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculated Mean or weighted MP |
| **Melting Point** | 10.93 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) US. Environmental Protection Agency. |

## 1.2 Boiling Point

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Boiling Point** | 110 °C |
| **Pressure** | 15 mm Hg |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **GLP** | No |
| **Boiling Point** | 224-228 °C |
| **Pressure** | 1013 hPa |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003a) Safety Data Sheet for 4-*tert*-butylcyclohexanol. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ASTM-D-1078 |
| **GLP** | No |
| **Boiling Point** | 223 °C |
| **Pressure** | 1013 hPa |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (1998a) Product information for 4-*tert*-butylcyclohexanol, Data Sheet 1391, 1.12. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculated. Adapted Stein & Brown method. |
| **Boiling Point** | 216.91 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |

| | |
|---|---|
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Boiling Point** | 216 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Boiling Point** | 212 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4.Only secondary literature (review, tables, books, etc.). |
| **References** | Merck Index (1997) Merck & Co., Inc. Whitehouse Station, NJ. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Boiling Point** | 260 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | ATSDM-D-1078 |
| **GLP** | No |
| **Boiling Point** | 241 °C |
| **Pressure** | 1013 hPa |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |

| | |
|---|---|
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (1998b) Product information for 4-*tert*-butylcyclohexyl acetate, Data Sheet 1401, 1.12. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **GLP** | No |
| **Boiling Point** | ca. 241 °C |
| **Pressure** | 1013 hPa |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003b) Safety Data Sheet for 4-*tert*-butylcyclohexyl acetate. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculated/Adapted Stein & Brown method |
| **Boiling Point** | 232.55 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

## 1.3 Vapor Pressure

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **GLP** | No |

| | |
|---|---|
| **Vapor Pressure** | Less than 0.1 hPa |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003a) Safety Data Sheet for 4-*tert*-butylcyclohexanol. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculated |
| **Vapor Pressure** | 0.005 mm Hg |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculated/Mean VP of Antoine & Grain methods |
| **Vapor Pressure** | 0.0263 mm Hg |
| **Temperature** | 25 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Vapor Pressure** | 0.02 mm Hg |

| | |
|---|---|
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **GLP** | No |
| **Vapor Pressure** | 0.01 hPa |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003b) Safety Data Sheet for 4-*tert*-butylcyclohexyl acetate. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculated |
| **GLP** | No |
| **Remarks for Substance** | The value at 20 °C is an estimate obtained by extrapolation of the data given in the reference: 241 °C: 1013 hPa, 200 °C: 366 hPa, 150 °C: 73 hPa, 100 °C: 8 hPa, log (VP) = -2866 * (1/T) + 8.6061 (T in K, VP in hPa) |
| **Vapor Pressure** | Approximately 0.067 hPa |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Huels AG (1985) Produktdatenblatt "p-tert.-Butylcyclohexylacetat", Artikel-Nr. 002304, 01-MAR-85. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |

| | |
|---|---|
| **Method/guideline** | Calculated |
| **Vapor Pressure** | 0.03 mm Hg |
| **Temperature** | 20 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Fragrance Materials Association (FMA) Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculated/Mean VP of Antoine & Grain methods |
| **Vapor Pressure** | 0.0159 mm Hg |
| **Temperature** | 25 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | MPBPVPWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

## 1.4 n-Octanol/Water Partition Coefficients

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | OECD Guideline 107 |
| **GLP** | No |
| **Year** | 1981 |
| **Remarks for Test Conditions** | Flask shaking method<br>GC analysis of the test substance<br>No further data |
| **Log Pow** | 3.23 |

| | |
|---|---|
| **Remarks for Results** | Summary report of results. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Degussa AG (1981) Unpublished Report No. 89-0514-DKP. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculated |
| **Log Pow** | 3.42 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (menthol) |
| **Method/guideline** | Calculated |
| **Log Pow** | 3.38 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity: 70.7% trans, 28.3 cis by GC |
| **Method/guideline** | Reverse phase HPLC method (OECD 117) |
| **Year** | 1996 |
| **Log Pow** | 4.8 at 25 °C |
| **Remarks for Results** | For both isomers |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Givaudan-Roure (1996) Partition coefficient n-octanol/water of 4-*tert*-butylcyclohexyl acetate. Unpublished. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculated |
| **Log Pow** | 4.42 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

## 1.5 Water Solubility

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **GLP** | No |
| **Value (mg/L) at Temperature** | Less than 100 mg/L at 20 °C |
| **Description of Solubility** | Of very low solubility |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003a) Safety Data Sheet for 4-*tert*-bytylcyclohexanol. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/Guideline** | Calculated |

| | |
|---|---|
| **Value (mg/L) at Temperature** | 528.9 at 25 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | WSKOWIN EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **GLP** | No |
| **Value (mg/L) at Temperature** | ca. 90 mg/L at 20 °C |
| **pH** | 7 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Only short abstract available. |
| **References** | Degussa AG (2003b) Safety Data Sheet for 4-*tert*-butylcyclohexyl acetate. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/Guideline** | Calculated |
| **Value (mg/L) at Temperature** | 2.552 at 25 °C |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | WSKOWIN EPI Suite (2000) U.S. Environmental Protection Agency. |

# 2 ENVIRONMENTAL FATE AND PATHWAYS

## 2.1 Photodegradation

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Calculation |
| **Test Type** | AOPWIN |
| **Halflife t1/2** | 6.361 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | AOPWIN EPI Suite (2000) U S Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Calculation |
| **Test Type** | AOPWIN |
| **Halflife t1/2** | 8.850 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | AOPWIN EPI Suite (2000) U S Environmental Protection Agency. |

## 2.2 Stability in Water

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method** | Calculated |
| **Halflife t 1/2** | 266 days at pH 8 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | HYDROWIN EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method** | Calculated |
| **Halflife t 1/2** | 7.2 years at pH 7 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | HYDROWIN EPI Suite (2000) US Environmental Protectin Agency. |

## 2.3 Biodegradation

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method** | EG-Guideline 84/449/EWG C.3 |
| **Test Type** | Aerobic |
| **GLP** | No |

| | |
|---|---|
| **Year** | 1983 |
| **Contact time (units)** | 19 days |
| **Innoculum** | Predominantly domestic sewage |
| **Remarks for Test Conditions** | Concentration: 20 mg/L related to DOC (dissolved organic carbon).<br>TEST CONDITION<br>- Additional substrate: No<br>- Test temperature: 20 +- 2°C<br>Kinetics: 5 d: 6%<br>14 d: 17%<br>19 d: 89 % |
| **Degradation % After Time** | 90% after 19 days |
| **Results** | Readily biodegradable |
| **Time required for 10% degradation** | Approximately 9 days |
| **10 day window criteria** | Fulfilled |
| **Total degradation** | 90 % |
| **Classification** | Readily biodegradable |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **Reference** | Degussa AG (1983) Unpublished report. Report No. 96-0464-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method** | EC-Guideline 92/69/E, CO2 Evolution test |
| **Test Type** | Aerobic |
| **GLP** | Yes |
| **Year** | 1992 |
| **Innoculum** | Activated sludge, domestic |
| **Remarks for Test Conditions** | INOCULUM<br>- Source: Kläranalge Marl Ost<br>Initial cell concentration: 122 x 10exp4 colony building units/L<br>INITIAL TEST SUBSTANCE CONCENTRATION: 20,6 to 20,8 |

| | |
|---|---|
| | mg/l<br>Test temperature: 20.4-23.6 °C<br>- pH value: 7.5-7.6<br>- suspended solids: 29.7 mg/l |
| **Degradation % After Time** | 75% after 28 days |
| **Results** | Readily biodegradable |
| **Time required for 10% degradation** | Approximately 5 days |
| **10 day window criteria** | Yes |
| **Total degradation** | 75% |
| **Classification** | Readily biodegradable |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **Reference** | Degussa AG (1997b) Unpublished report. Report No, 97-0300-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method** | ISO Draft "BOD Test for insoluble substance" |
| **Test Type** | Aerobic |
| **GLP** | No |
| **Year** | 1995 |
| **Contact time (units)** | 28 days |
| **Innoculum** | Activated sludge, domestic |
| **Remarks for Test Conditions** | INNOCULUM<br>- Source: Lippeverbands-Kläranlage Marl<br>INITIAL TEST SUBSTANCE CONCENTRATION: 51.5 mg/l<br>DURATION OF THE TEST: 28 days<br>ANALYTICAL PARAMETER: Oxygen consumption<br>SAMPLING:on days 0, 7, 14, 21, 28<br>Control: Diethjylene glycol |
| **Degradation % After Time** | 68% after 28 days |
| **Time required for 10% degradation** | Approximately 10 days |

| | |
|---|---|
| **10 day window criteria** | Not met |
| **Total degradation** | 68 % |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **Reference** | Degussa AG (1995) Unpublished report. Report No. 95-0220-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity: 70.8% trans, 28.3% cis by GC |
| **Method** | Respirometric Method, modified MITI test II (OECD Guideline No. 302 C) |
| **GLP** | Yes |
| **Year** | 1995 |
| **Contact time (units)** | Up to 28 days |
| **Innoculum** | Fresh activated sludge, domestic sewage |
| **Remarks for Test Conditions** | Dry weight of suspended solids=3.980 g/L; sludge concentration= 100 mg/L (d.w.); nominal concentration=30 mg/L; temperature=22 deg C; biodegradation began on day 12 |
| **Degradation % After Time** | 24% after 28 days |
| **Results** | Not inherently but partially biodegradable |
| **Time required for 10% degradation** | 12-14 days |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **Reference** | Rudio J. (1996b) Inherent biodegradability of 4-*tert*-butylcyclohexyl acetate according to OECD Guideline No. 302 C. Givaudan Roure Test Report No. 96-E51. Unpublished, dated June 24, 1996. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity: 70.8% trans, 28.3% cis by GC |
| **Method** | Manometric Respirometric Test (OECD Guideline No. 301 F) |
| **Test Type** | Yes |

| | |
|---|---|
| **GLP** | Yes |
| **Year** | 1996 |
| **Contact time (units)** | Up to 28 days |
| **Innoculum** | Fresh activated sludge, domestic sewage |
| **Remarks for Test Conditions** | Dry weight of suspended solids=4.366 g/L; sludge concentration= 30 mg/L (d.w.); nominal concentration=100 mg/L; temperature=22 deg C; biodegradation began on day 15 |
| **Degradation % After Time** | 54% after 28 days |
| **Results** | Not readily biodegradable |
| **Time required for 10% degradation** | 15 days |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **Reference** | Rudio J. (1996a) Ready biodegradability of butylcyclohexyl acetate according to OECD Guideline No. 301 F. Givaudan Roure. Unpublished, dated January 17, 1996. |

## 2.4 Fugacity

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Method** | Mackay |
| **Model Used** | Level III Fugacity-based Environmental Equilibrium Partitioning Model |
| **Input Parameters** | MW, calculated VP, calculated MP, calculated Kow |
| **Year** | 2000 |
| **Model data and results** | Air = .92%<br>Water = 37.9%<br>Soil = 59.7%<br>Sediment = 0.468% |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Mackay D. (1991) Multimedia Environmental Models; The Fugacity Approach, Lewis Publishers, CRC Press, pp 67-183.<br>Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five-stage process. Environmental Toxicology and Chemistry, 15(9), 1618-1626.<br>Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Model Conditions** | Mackay |
| **Test Type** | Environmental Equilibrium Partitioning Model |
| **Model Used** | Level III Fugacity-based Environmental Equilibrium Partitioning Model |
| **Input Parameters** | MW, calculated VP, calculated MP, calculated Kow |
| **Year** | 2000 |
| **Model data and results** | Air = 1.66%<br>Water = 14.9%<br>Soil = 71.3%<br>Sediment = 12.1% |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **References** | Mackay D. (1991) Multimedia Environmental Models; The Fugacity Approach, Lewis Publishers, CRC Press, pp 67-183.<br>Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five-stage process. Environmental Toxicology and Chemistry, 15(9), 1618-1626.<br>Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637. |

# 3 ECOTOXICITY

## 3.1 Acute Toxicity to Fish

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | DIN 38412 part 15 |
| **Test Type** | Static |
| **GLP** | No |
| **Year** | 1987 |
| **Species/Strain/Supplier** | Fish/*Leuciscus idus* (Fresh water) |
| **Exposure Period** | 48 hours |
| **Analytical monitoring** | No |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Leuciscus idus melanotus*<br>- Supplier: Fischzucht Eggers / Hohenwestedt<br>- Size/weight/loading: 6 cm +- 2 cm / 10<br>- Feeding: Tetramin before start of test<br>- Pretreatment: 14 d prior to experiment, Zephirol 1: 1000 for 1 hour<br>- Feeding during test: no<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>- Vehicle, solvent: no<br>STABILITY OF THE TEST CHEMICAL SOLUTIONS: stable<br>REFERENCE SUBSTANCE: no<br>DILUTION WATER<br>- Source: decholrinated drinking water<br>- Aeration: continuously<br>- Hardness: no data<br>- pH: 7.9 to 8.3<br>- Oxygen content: 8.3 to 9.5 mg/l |
| **Unit** | mg/L |
| **Remarks fields for results** | RESULTS: |

| | |
|---|---|
| | - Nominal/measured concentrations: nominal only:<br>13, 16, 20 mg/l<br>- Effect data (Mortality):<br>13 mg/l: 0%<br>16 mg/l: 20%<br>20 mg/l: 100 % |
| **Conclusion Remarks** | LC50 = 17 mg/L<br>LC0 = 13 mg/l<br>LC100= 20 mg/l |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **Reference** | Degussa AG (1987) Unpublished report. Report No. 96-0394-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Fish |
| **Exposure Period** | 96 hour |
| **Remarks for Test Conditions** | Based on: log KOW = 3.23, MP = 67 °C, water solubility = 100 mg/L |
| **Unit** | mg/L |
| **Endpoint value** | LC50 = 8.085 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Fish |

| | |
|---|---|
| **Exposure Period** | 14 days |
| **Remarks for Test Conditions** | Based on: log KOW = 3.23, MP = 67 °C, water solubility = 100 mg/L |
| **Unit** | mg/L |
| **Endpoint value** | LC50 = 17.805 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | DEV DIN 38412 part 15 |
| **Test Type** | Static |
| **GLP** | No |
| **Year** | 1985 |
| **Species/Strain/Supplier** | Fish/*Leuciscus idus*, fresh water |
| **Exposure Period** | 48 hours |
| **Analytical monitoring** | No |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Leuciscus idus melanotus*<br>- Supplier: Fa. Ekkerts<br>- Loading: 10 per vial<br>- Feeding: Tetramin<br>- Pretreatment: Zephirol 1: 50000<br>- Feeding during test: no<br>DILUTION WATER<br>- Source: Drinking water dechlorinated<br>Temperature: 20+-1°C<br>pH: 7.6-7.8<br>Hardness: 15°dH<br>Dissolved oxygen: 7.3-8.1 mg/l<br>Statistical analysis: Linear regression |
| **Nominal concentrations as mg/L** | 10 to 20 |

| | |
|---|---|
| **Unit** | mg/L |
| **Endpoint value** | Mortality |
| **Remarks fields for results** | Because of the low solubility of the test substance a solubilizer was used Marlowet EF.<br>RESULTS:<br>- Nominal concentrations (mg/l): Mortality %<br>10 0<br>13 10<br>16 80<br>20 100<br>- Concentration / response curve: slope: 4.8, correlation coefficient: 0.98<br>LC50: 14 mg/l<br>95 %Confidence limit: 11 - 19 mg/l |
| **Conclusion Remarks** | LC0 = 10 mg/L, LC50 = 14 mg/L, LC100 = 20 mg/L<br>Confidence limit: 11-19 mg/L. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **Reference** | Degussa AG (1985a) Unpublished report. Report. No. 85-0370-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity 99.1 % |
| **Method/guideline** | CE Guideline 92/69/EEC, part C1 |
| **Test Type** | Semistatic |
| **GLP** | Yes |
| **Year** | 1992 |
| **Species/Strain/Supplier** | Fish/*Cyprinus carpio*, fresh water |
| **Exposure Period** | 96 hours |
| **Analytical monitoring** | Yes |
| **Remarks for Test Conditions** | As the test substance was not readily soluble in water, a suspension of 1 g test substance/L was stirred for 18 hours. After that a filtrate of the suspension was used for the test. |
| **Nominal concentrations as mg/L** | 4.0 to 34 |

| | |
|---|---|
| **Measured concentrations as mg/L** | 4.0 to 34 |
| **Unit** | mg/L |
| **Endpoint value** | Death |
| **Remarks fields for results** | Temperature: ca. 20 °C<br>Dissolved oxygen: 85-100% saturation<br>pH: 8.0-8.4<br>Hardness: 12.5-13 degree dH |
| **Conclusion Remarks** | LC0 = 6.7 mg/L, LC50 = 8.6 mg/L, LC100 = 12 mg/L. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **Reference** | Degussa AG (1997c) Unpublished report. Report No. 97-0304-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Fish |
| **Exposure Period** | 96 hour |
| **Remarks for Test Conditions** | Based on: log KOW = 4.80, water solubility = 90 mg/L |
| **Unit** | mg/L |
| **Endpoint value** | LC50 = 0.954 |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency. |

## 3.2 Acute Toxicity to Aquatic Invertebrates

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Purity: 99 % |
| **Method/guideline** | EC Guideline 92/69/EEC |
| **Test Type** | Experimental |
| **GLP** | Yes |
| **Year** | 1992 |
| **Analytical procedures** | TOC analysis |
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Daphnia magna*<br>- Source/supplier: Hüls AG Prüfinstitut für Biologie, Marl, Germany<br>- Breeding method: in M4 medium (Elendt, 1990), 1 l bakers<br>- Age: < 24 h<br>- Feeding: *Scenedesmus subspicatus*<br>- Pretreatment: no<br>- Feeding during test: no<br>- Control group: yes<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>- Dispersion: 1 g/l in synthetic fresh water, mixing over 18 hours, filtration, DOC determination.<br>Reference substance: potassium dichromate<br>STABILITY OF THE TEST CHEMICAL SOLUTIONS: stable<br>DILUTION WATER<br>- Source: synthetic fresh water<br>CaCl2 x 2 H2O: 294 mg/l<br>MgSO4 x 7 H2O: 123 mg/l<br>NaHCO3: 63 mg/l<br>KCl: 5.5 mg/l<br>- Aeration: no |

| | |
|---|---|
| | - Hardness: Ca2+ and Mg2+: 2.5 mmol/l<br>- TOC: 0<br>- Ca/Mg ratio: 4:1<br>- Na/K ratio: 10:1<br>- pH: 7.7 to 7.8<br>- Oxygen content: 7.9 to 8.1 mg/l |
| **Measured concentrations as mg/L** | Less than 20 % deviation from nominal conc. |
| **EC50, EL50, LC0, at 24,48 hours** | EC0 = 25, EC50 = 46, EC100 = 75 mg/L. |
| **Biological observations** | EC50 (24 h): 46 mg/l<br>EC50 (48 h): 46 mg/l |
| **Control response satisfactory?** | Yes |
| **Appropriate statistical evaluations?** | Yes |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Data Reliability Remarks** | Code 1. Comparable to guideline study. |
| **Reference** | Degussa AG (1994b) Unpublished report. Report No.: 94-0228-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | *Daphnia Magna* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 3.23, MP = 67 °C, water solubility = 100 mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | LC50 = 9.431 at 48 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Data Reliability Remarks** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |

| | |
|---|---|
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Mysid shrimp |
| **Test Details** | 96 hours |
| **Remarks for Test Conditions** | Based on: log KOW=3.23, MP=67 °C, water solubility=100 mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | LC50 = 0.969 at 96 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Data Reliability Remarks** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity 99.1% |
| **Method/guideline** | EG EG92/69/EWG |
| **Test Type** | Static |
| **GLP** | Yes |
| **Year** | 1997 |
| **Analytical procedures** | DOC |
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Daphnia magna*<br>- Source/supplier: Hüls AG Prüfinstitut für Biologie, Marl, Germany<br>- Breeding method: in M4 medium (Elendt, 1990), 1 l bakers<br>- Age: < 24 h<br>- Feeding: *Scenedesmus subspicatus*<br>- Pretreatment: no<br>- Feeding during test: no<br>- Control group: yes<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION |

| | |
|---|---|
| | - Dispersion: 1 g/l in synthetic fresh water, mixing over 18 hours, filtration, DOC determination.<br>Reference substance: potassium dichromate<br>STABILITY OF THE TEST CHEMICAL SOLUTIONS: stable<br>DILUTION WATER<br>- Source: synthetic fresh water<br>CaCl2 x 2 H2O: 294 mg/l<br>MgSO4 x 7 H2O: 123 mg/l<br>NaHCO3: 65 mg/l<br>KCl: 6 mg/l<br>- Aeration: no<br>- Hardness: Ca2+ and Mg2+: 2.5 mmol/l<br>- TOC: 0<br>- Ca/Mg ratio: 4:1<br>- Na/K ratio: 10:1<br>- pH: 7.7 to 7.8<br>- Oxygen content: 8.9 to 9.1 mg/l<br>Temperature: 20+-1°C |
| **Nominal concentrations as mg/L** | 2.8-28-4 |
| **Measured concentrations as mg/L** | 2.4-28-4 |
| **EC50, EL50, LC0, at 24,48 hours** | EC50 = 23.4, EC10 = 8.7, EC100 > 28.1 |
| **Control response satisfactory?** | Yes |
| **Appropriate statistical evaluations?** | Yes |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Data Reliability Remarks** | Code 1. Guideline study. |
| **Reference** | Degussa AG (1997a) Unpublished report. Report No. 97-0302-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | DIN 38412 part 11 |
| **Test Type** | Static |
| **GLP** | No |

| | |
|---|---|
| **Year** | 1985 |
| **Analytical procedures** | DOC |
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 24 hour |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Daphnia magn*a<br>- Supplier: Hüls AG<br>- Age/loading: < 1 d, 5<br>- Feeding: Chlorella Vulgaris<br>- Feeding during test: no<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>- Stock solution: in water, filtration after 2 h of stirring.<br>Dilution water: synthetic: Cacl2 x 2 H2O: 294 mg/l<br>MgSO4 x 7 H2O 123 mg/l<br>NaHCO3 63 mg/l<br>KCl 5,5 mg/l<br><br>- Test temperature: 20 +- 1 °C<br>- Hardness: 2.5 mmol Ca2+ and Mg2+, Ca2+ : Mg2+: 4: 1<br>DURATION OF THE TEST: 24 h<br>TEST PARAMETER: Immobilisation<br><br>MONITORING OF TEST TEST ORGANISMS<br>- Strain: *Daphnia magna*<br>- Supplier: Hüls AG<br>- Age/loading: < 1 d, 5<br>- Feeding: Chlorella Vulgaris<br>- Feeding during test: no<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>- Stock solution: in water, filtration after 2 h of stirring.<br>Dilution water: synthetic: Cacl2 x 2 H2O: 294 mg/l<br>MgSO4 x 7 H2O 123 mg/l<br>NaHCO3 63 mg/l<br>KCl 5,5 mg/l<br><br>- Test temperature: 20 +- 1 °C |

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|---|---|
| | - Hardness: 2.5 mmol Ca2+ and Mg2+, Ca2+ : Mg2+: 4: 1<br>DURATION OF THE TEST: 24 h<br>TEST PARAMETER: Immobilisation<br><br>MONITORING OF TEST SUBSTANCE CONCENTRATION: yes by DOC |
| **Measured concentrations as mg/L** | 2,6 to 22 |
| **EC50, EL50, LC0, at 24,48 hours** | 24 h EC50 = 7 mg/l, EC10= 2.6 mg/l EC100= 22 mg/l |
| **Biological observations** | Immobilization |
| **Appropriate statistical evaluations?** | Regression analysis. |
| **Remarks for results** | Results based on DOC in mg/l. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Data Reliability Remarks** | Code 2. Basic data given: comparable to guidelines/standards. |
| **Reference** | Degussa AG (1985b) Unpublished report. Report No. 85-0368-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | DIN 38412 part 11 |
| **Test Type** | Static |
| **GLP** | No |
| **Year** | 1985 |
| **Analytical procedures** | No |
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 24 hours |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Strain: *Daphnia magna*<br>- Supplier: Hüls<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>Solution using a solubiliser: Marlowet ef<br>- Concentrations: 12, 18, 25, 35 mg/l<br>DURATION OF THE TEST: 24 hours<br>TEST PARAMETER: Immobilisation |

| | |
|---|---|
| **Nominal concentrations as mg/L** | 12-35 |
| **EC50, EL50, LC0, at 24,48 hours** | EC50 = 19, EC0=12, EC100= 35 |
| **Appropriate statistical evaluations?** | Regression analysis |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Data Reliability Remarks** | Code 2. Basic data given: comparable to guidelines/standards. |
| **Reference** | Degussa AG (1985c) Unpublished report. Report No. 85-0378-DKO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | *Daphnia magna* |
| **Test Details** | 48 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 4.80, water solubility = 90 mg/L |
| **EC50, EL50, LC0, at 24,48 hours** | LC50 = 0.446 at 48 hours |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Data Reliability Remarks** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) US Environmental Protection Agency. |

## 3.3 Acute Toxicity to Aquatic Plants

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | 99.3 % purity (GC) |
| **Method/guideline** | EC 92/69/EEC |

| | |
|---|---|
| **Test Type** | Biomass/growth rate |
| **GLP** | Yes |
| **Year** | 1992 |
| **Species/Strain/Supplier** | *Scenedesmus subspicatus* (algae) |
| **Endpoint Value** | Growth |
| **Exposure Period** | 72 hour |
| **Analytical monitoring** | Yes |
| **Remarks for Test Conditions** | - Initial cell concentration: 20000 cells/ml<br>STOCK AND TEST SOLUTION AND THEIR PREPARATION<br>- Dispersion: 1 g/l in synthetic fresh water, mixing over 18 hours, filtration, DOC determination. |
| **Nominal concentrations as mg/L** | 4.4 to 148 |
| **Measured concentrations as mg/L** | 4.6 to 110 |
| **Unit** | mg/L |
| **Endpoint value** | Growth |
| **NOEC, LOEC or NOEL, LOEL** | NOEC=14, EC10=15, and EC50= 29 mg/L. |
| **Biological observations** | EC50 for growth rate: 45 mg/l<br>EC10 for growth rate: 21 mg/l |
| **Control response satisfactory?** | Yes |
| **Appropriate statistical evaluations?** | Yes |
| **Conclusion remarks** | EC50 (72 h, growth rate) = 45 mg/L<br>EC10 (72 h, growth rate} = 21 mg/L |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **Reference** | Degussa AG (1994a) Unpublished report. Report No.: 94-0230-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |

| | |
|---|---|
| **Species/Strain/Supplier** | Green algae |
| **Exposure Period** | 96 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 3.23, MP = 67 C, water solubility = 100 mg/L |
| **Unit** | mg/L |
| **NOEC, LOEC or NOEL, LOEL** | EC50 = 6.329 mg/L |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) US Environmental Protection Agency. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Remarks for Substance** | Purity 99.1% |
| **Method/guideline** | EC Guideline 92/69/EEC |
| **GLP** | Yes |
| **Year** | 1992 |
| **Species/Strain/Supplier** | *Scendesmus subspicatus* |
| **Endpoint Value** | Biomass and growth rate |
| **Exposure Period** | 72 hour |
| **Analytical monitoring** | Yes |
| **Remarks for Test Conditions** | As the test substance was not readily soluble in water, suspension of 1 g test substance/L was stirred for 18 hours. After that a filtrate of the suspension was used for the test.<br>Temperature: 23.3-23.8 °C;<br>pH: 7.5-9.3 |
| **Nominal concentrations as mg/L** | 0.76 to 27.3 |
| **Measured concentrations as mg/L** | 0.76 to 27.3 |
| **Unit** | mg/L |
| **NOEC, LOEC or NOEL, LOEL** | NOEC = 6.8, EC10 = 8.2, EC50 = 17 mg/L |
| **Control response satisfactory?** | Yes |
| **Appropriate statistical evaluations?** | Yes |

| | |
|---|---|
| **evaluations?** | |
| **Conclusion Remarks** | Effect concentrations based on growth rate<br>EC10 = 11 mg/L<br>EC50 = 22 mg/L |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **Reference** | Degussa AG (1992) Unpublished report. Report No. 97-0308-DGO. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | ECOSAR |
| **Test Type** | Calculated |
| **Species/Strain/Supplier** | Green algae |
| **Exposure Period** | 96 hours |
| **Remarks for Test Conditions** | Based on: log KOW = 4.80, water solubility = 90 mg/L |
| **Unit** | mg/L |
| **NOEC, LOEC or NOEL, LOEL** | EC50 = 0.084 mg/L |
| **Data Qualities Reliabilities** | Reliability code 4. Not assignable. |
| **Remarks for Data Reliability** | Code 4. Calculated. |
| **Reference** | ECOSAR EPI Suite (2000) US Environmental Protection Agency. |

# 4 HUMAN HEALTH TOXICITY

## 4.1 Acute Toxicity

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Test Type** | Acute Oral LD50 |
| **GLP** | Yes |
| **Year** | 1973 |
| **Species/strain** | Rat |
| **# of animals per sex per dose** | 10 |
| **Route of Administration** | Oral |
| **Value LD50 or LC50 with confidence limits** | LD50 = 4200 mg/kg bw; Confidence limits 3620 - 4870 mg/kg. |
| **Number of deaths at each dose level** | 95% confidence limit = 3620-4870 mg/kg. Toxic signs were immediate stimulation followed by ataxia. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Denine E.P. and Palanker A.C. (1973) Acute oral and dermal toxicity studies. Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Acute Toxicity LD50 |
| **GLP** | No |
| **Year** | 1962 |
| **Species/strain** | Mouse/CF1 |
| **Route of Administration** | Intraperitoneal |
| **Remarks for Test Conditions** | Groups of 10 adult CF1 strain mice weighing 20-25 grams (6-8 weeks old) were injected with test compound and the resulting mortality was recorded for one week. The mice were housed 10 per cage in an air-conditioned room (75 to 80 F) and were provided food and water ad libitum. Vehicle was distilled H2O |

| | |
|---|---|
| | when possible. Compounds insoluble in H2O were dissolved in mixtures of H2O and propylene glycol or cottonseed oil or suspended in 0.5% solution of carboxymethylcellulose. |
| **Value LD50 or LC50 with confidence limits** | LD50 = 50-100 mg/kg. |
| **Conclusion remarks** | LD50 = 50-100 mg/kg. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Doull J., V.Plzak and S.J.Brois. (1962) A survey of compounds for radiation protection. Unpublished report. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Test Type** | Acute Dermal LD50 |
| **GLP** | No |
| **Year** | 1973 |
| **Species/strain** | Rabbit |
| **# of animals per sex per dose** | 6 |
| **Route of Administration** | Dermal |
| **Value LD50 or LC50 with confidence limits** | LD50 = greater than 5000 mg/kg bw |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Denine E.P. and Palanker A.C. (1973) Acute oral and dermal toxicity studies. Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Litchfield and Wilcoxon, 1949 |
| **Test Type** | Acute Oral LD50 |
| **GLP** | No |
| **Year** | 1964 |

| | |
|---|---|
| **Species/strain** | Rat/Osborne-Mendel |
| **Sex** | Male and Female |
| **# of animals per sex per dose** | 5 |
| **Vehicle** | Corn oil |
| **Route of Administration** | Oral-Gavage |
| **Value LD50 or LC50 with confidence limits** | 3180 mg/kg bw (2790-3620) |
| **Remarks for Results** | Slope function:<br>1.3 (95% CL 1.1-1.5) |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. Fd Cosmet Toxicol 2:327-343. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Reed-Munch method |
| **Test Type** | Acute Oral LD50 |
| **GLP** | No |
| **Year** | 1975 |
| **Species/strain** | Mouse |
| **Sex** | Male |
| **# of animals per sex per dose** | 6 |
| **Route of Administration** | Oral |
| **Remarks for Test Conditions** | Doses given ranged from 2000 to 5000 mg/kg bw and given as a 37.5% (w/v) emulsion. |
| **Value LD50 or LC50 with confidence limits** | 4384 mg/kg bw |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |

| | |
|---|---|
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Litchfield and Wilcoxon, 1949 |
| **Test Type** | Acute Oral LD50 |
| **GLP** | No |
| **Year** | 1975 |
| **Species/strain** | Rat |
| **Sex** | Male |
| **# of animals per sex per dose** | 5 |
| **Vehicle** | 0.85% saline |
| **Route of Administration** | Oral-Gavage |
| **Value LD50 or LC50 with confidence limits** | 940 mg/kg bw (95% CL=534-1654) |
| **Number of deaths at each dose level** | 250 mg/kg bw: 0/5<br>500 mg/kg bw: 1/5<br>1000 mg/kg bw: 3/5<br>2000 mg/kg bw: 4/5<br>3000 mg/kg bw: 5/5 |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Test Type** | Acute Oral LD50 |
| **Year** | 1975 |

| | |
|---|---|
| **Species/strain** | Rat |
| **# of animals per sex per dose** | 10 |
| **Route of Administration** | Oral |
| **Value LD50 or LC50 with confidence limits** | LD50 = 5000 mg/kg. |
| **Remarks for Results** | Acute oral LD50 approximately equals 5000 mg/kg. Toxic signs were lethargy, tremors and chromodacryorrhea. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Moreno O.M. (1976) Acute toxicity studies in rats, mice, rabbits and guinea pigs. Unpublished report to RIFM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Test Type** | Acute Oral LD50 |
| **GLP** | No |
| **Year** | 1970 |
| **Species/strain** | Rat |
| **Sex** | Not reported |
| **Vehicle** | Traganth |
| **Route of Administration** | Oral |
| **Remarks for Test Conditions** | Substance administered as a 2-30% emulsion |
| **Value LD50 or LC50 with confidence limits** | Approximately 4,800 ml/kg bw |
| **Remarks for Results** | Signs included dyspnea, shivering, cramping. At necropsy, rats showed bloody snouts and anus, diarrhea, enlarged gastrointestinal tract and bladder. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Zeller and Hofmann (1970) p-*tert*-Butylcyclohexylacetat. Ergebnis der Gewerbetoxikologischen Vorpruefung. Dated 21.7.1970. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |

| | |
|---|---|
| **CAS No.** | 32210-23-4 |
| **Test Type** | Acute Oral LD50 |
| **GLP** | No |
| **Year** | 1976 |
| **Species/strain** | Rat |
| **Sex** | Not reported |
| **# of animals per sex per dose** | 10/dose |
| **Route of Administration** | Oral |
| **Remarks for Test Conditions** | Two doses administered: 500 or 5000 mg/kg bw |
| **Value LD50 or LC50 with confidence limits** | Less than 5 but greater than 500 mg/kg bw |
| **Number of deaths at each dose level** | 500 mg/kg bw: 3 deaths/10 rats<br>5000 mg/kg bw: 8 deaths/10 rats |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Opdyke, D.L. (1976) Acute oral toxicity in rats, dermal toxicity in rabbits. Report to RIFM dated May 21, 1976. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Test Type** | IP LD50 |
| **GLP** | No |
| **Year** | 1970 |
| **Species/strain** | Mouse |
| **Sex** | Not reported |
| **Vehicle** | Traganth |
| **Route of Administration** | Intraperitoneal |
| **Remarks for Test Conditions** | Substance administered as a 2-30% emulsion |
| **Value LD50 or LC50 with confidence limits** | Approximately 400 ml/kg bw |
| **Remarks for Results** | Signs included dyspnea, shivering, cramping. At necropsy, lesions were reported in the forestomach. |

| | |
|---|---|
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Zeller and Hofmann (1970) p-*tert*-Butylcyclohexylacetat. Ergebnis der Gewerbetoxikologischen Vorpruefung. Dated 21.7.1970. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Test Type** | Acute Dermal LD50 |
| **GLP** | No |
| **Year** | 1976 |
| **Species/strain** | Rabbit |
| **Sex** | Not reported |
| **# of animals per sex per dose** | 4/dose |
| **Route of Administration** | Dermal |
| **Remarks for Test Conditions** | One dose of 5000 mg/kg bw administered. Insufficient test material to dose 10 rabbits. |
| **Value LD50 or LC50 with confidence limits** | Greater than 5000 mg/kg bw |
| **Number of deaths at each dose level** | 5000 mg/kg bw: 1 death/5 rabbits |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Opdyke, D.L. (1976) Acute oral toxicity in rats, dermal toxicity in rabbits. Report to RIFM dated May 21, 1976. |

## 4.2 Genetic Toxicity

### 4.2.1 *In vitro* Genotoxicity

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Test Type** | Ames test |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1988 |
| **Species/Strain** | *Salmonella typhimuriums* TA98, TA 100, TA 1535, TA 1537, TA 1538 |
| **Metabolic Activation** | With and without |
| **Doses/Concentration** | Up to 5000 ug/plate |
| **Remarks for Test Conditions** | SYSTEM OF TESTING<br>- Metabolic activation system: Arochlor induced rat liver S9-mix of Bor: W/SW male rats (source SPF, TNO, NL)<br>- No. of metaphases analyzed: no indicated<br>- concentrations: 10 to 5000 micro-g/plate<br>- Number of replicates: 2 one plate incorporation on pre-incubation test.<br>- Positive and negative control groups and treatment:<br>Negative: Solvent, DMSO<br>Positive: TA 98, TA 1528: Nitrofluorene, TA 100, TA 1535: Sodiumazide, TA 1537: Aminocridine<br>Cytotoxicity: from 250 or 500 micro-g per plate |
| **Results** | Negative |
| **Cytotoxic concentration** | 250 to 500 micrograms/plate |
| **Genotoxic Effects** | None |
| **Remarks for results** | GENOTOXIC EFFECTS:<br>- With metabolic activation: none<br>- Without metabolic activation: none<br>CYTOTOXIC CONCENTRATION:<br>- With metabolic activation: 500 ug/plate |

| | |
|---|---|
| | - Without metabolic activation: 250 ug/plate<br>TEST-SPECIFIC CONFOUNDING FACTORS: none<br>STATISTICAL RESULTS: not given |
| **Conclusion Remarks** | Not mutagenic with and without metabolic activation |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Degussa AG (1988a) Unpublished report. Reg.Nr.: 88-0660-DKM. |

| | |
|---|---|
| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | Ames test |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Ames reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1982 |
| **Species/Strain** | *Salmonella typhimurium* strains TA100, TA2637, and TA98 |
| **Metabolic Activation** | S9 mix |
| **Doses/Concentration** | 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg/plate |
| **Remarks for Test Conditions** | DMSO used as solvent control. |
| **Results** | No increase in his+ revertant frequency at any concentration tested. |
| **Cytotoxic concentration** | 0.5 mg/plate for all strains; 0.2 mg/plate for TA100 -S9 |
| **Genotoxic Effects** | None |
| **Remarks for Results** | Foreign paper, data taken from tables and brief English summary. |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol was not mutagenic when tested in Salmonella typhimurium strains TA100, TA2637 or TA98 with or without metabolic activation. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Nohmi, T., Miyatea, R., Yoshikawa, K., and Ishidate, M. Jr. (1985) Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity |

tests. Bull Natl Inst Hyg Sci 103:60-64.

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Preincubation assay (Haworth *et al.,* 1983) |
| **Test Type** | Ames reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | Ambiguous |
| **Year** | 1988 |
| **Species/Strain** | *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat and male Syrian hamster |
| **Doses/Concentration** | 0, 3, 10, 33, 100, 166, 333, and 666 ug/plate |
| **Remarks for Test Conditions** | At least 5 concentrations of test chemical tested in triplicate with metabolic activation (10% and 30% of rat or hamster S9) and without S9. Positive controls used were sodium azide, 9-aminoacridine, and 4-nitro-o-phenylenediamine. DMSO used as solvent control. |
| **Results** | No increase seen in number of his+ revertants with or without any type/concentration of S9 at any of the concentrations tested. |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol was not mutagenic when tested in Salmonella typhimurium strains TA100, TA1535, TA97, or TA98 with or without metabolic activation. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. Part of NTP study program. |
| **References** | Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988) Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl 12): 1-158.<br><br>Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E. (1983) Salmonella mutagenicity results for 250 chemicals. Environ Mutagen 5(Suppl 1): 3-142. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Ames test |
| **Test Type** | Ames reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1984 |
| **Species/Strain** | *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98 |
| **Metabolic Activation** | S9 fraction from liver of PCB-induced Fischer rats |
| **Doses/Concentration** | Max. Concentration = 5.0 mg/plate |
| **Remarks for Test Conditions** | Overnight cell cultures were preincubated at 37 °C with the test chemical and S9 for 20 minutes prior to plating. Six concentrations of the test chemical were tested in duplicate. The number of revertants was scored after the plates were incubated for 2 days at 37 °C. A chemical was considered mutagenic if the number of revertants was 2X the number of colonies in the solvent control. DMSO was the solvent control. |
| **Results** | No increase in the number of revertants. |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol was not mutagenic when tested in Salmonella typhimurium strains TA92, TA1535, TA100, TA1537, TA94, or TA98 with metabolic activation. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., and Matsuoka, A. (1984). Primary mutagenicity screening of food additives currently used in Japan. Fd Chem Toxic 22(8): 623-636. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | OECD Guideline 473 |
| **Test Type** | Cytogenetic assay |

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| **System of Testing** | Non bacterial |
| **GLP** | Yes |
| **Year** | 1997 |
| **Species/Strain** | Hamster/Chinese V79 lung cells |
| **Metabolic Activation** | With and without |
| **Doses/Concentration** | With metabolic activation: 20 - 500 micrograms/ml, without metabolic activation 20 - 200 micrograms/ml |
| **Statistical Methods** | For testing acceptable homogeneity between replicates: Binominal Dispersion Test (Richardson et al., 1989).<br>Comparison of structural aberrations between treated and controls: Chi-square Test ($p < 0.05$). |
| **Remarks for Test Conditions** | Cytotoxicity screening test between 10 to 2000 micrograms/ml, solubility limit in Ethanol 1%.<br>SYSTEM OF TESTING<br>- Cell type: Chinese Hamster lung cells V79<br>- Metabolic activation system: Arochlor induced liver S9 fraction of male Spraque Dawley Rats.<br>- No. of metaphases analyzed: 100 from each culture:<br>- Cocentrations: without S9: 10, 60, 100 micro g/ml (test 1 and 2)<br>with S9: 50, 250, 500 (test 1) and 20, 100, 200 micro g/ml (test 2)<br>- Number of replicates: 2 per culture, 2 independent experiments.<br>- Positive and negative control groups and treatment:<br>Negative control: MEM 4 medium with and without S9 mix.<br>Positive controls:<br>Without metabolic activation: Mitomycin C 0.03 to 0.04 micro g/ml<br>With metabolic activation:<br>Cyclophosphamide, 3 and 4 micro g/ml<br>Harvest time: 18 and 28 hours<br>CRITERIA FOR EVALUATING RESULTS:<br>The chemical is regarded as clastogenic if:<br>- it induces chromosomal aberrations in a statistically significant manner in one or more concentrations.<br>- the induced proportion of aberrant cells at such test substance concentrations exceeds the normal rang of the test system (greater than 5%).<br>- positive results can be verified in an independent experiment. |

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| | STATISTICAL METHODS:<br>For testing acceptable homogeneity between replicates: Binominal Dispersion Test (Richardson et al., 1989).<br>Comparison of structural aberrations between treated and controls: Chi-square Test ($p < 0.05$). |
| **Results** | Negative.<br>No biologically significant increases in chromosomal aberrations (excluding gaps} in both experiments with and without metabolic activation at both sampling times. A statistically significant increase in the highest concentration with S9 at the 28 hour sampling time was within the normal range of chromosomal aberrations of the test system and related to the low control incidence of the experiment (0%) . As no dose related increase in chromosomal aberrations could be detected in the other experiments, this result was not considered biologically significant. 4-*tert*-Butylcyclohexanol was not clastogenic in this experiment. |
| **Cytotoxic concentration** | Without S9 mix 60 to 100 ug/ml, with S9 mix 200 to 500 ug/ml |
| **Genotoxic Effects** | None detected |
| **Appropriate statistical evaluations?** | Yes |
| **Conclusion Remarks** | The test substance was not clastogenic under the described experimental conditions |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | Degussa AG (1997) Unpublished report. Report No. 97-0366-DGM. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Chromosomal Aberration assay |
| **System of Testing** | Non bacterial |
| **GLP** | Ambiguous |
| **Year** | 1991 |
| **Species/Strain** | Human lymphocyte |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat |
| **Doses/Concentration** | 0.1, 1.0, 10 mM with and without S9 |

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| **Statistical Methods** | Chi-square test |
| **Remarks for Test Conditions** | Lymphocytes were obtained from healthy donors (12 of each sex) and cultured at ~0.5-1.0E6 isolated cells. Cultures were incubated in the dark at 37 °C for 72 hours after which cells were exposed for 1 hour to colchicine and slides were prepared and chromosomal aberrations were scored in 100 metaphase cells. Solvent control was DMSO and positive control was MMC. |
| **Results** | Total structural aberrations (no./100 cells) for DMSO, DMSO+S9, MMC, 0.1 mM, 0.1 mM+S9, 1.0 mM, 1.0 mM+S9, 10 mM, and 10 mM+S9 was 1.76, 2.00, 9.13, 1.90, 2.03, 2.18, 2.02, 2.11 and 2.23, respectively. |
| **Appropriate statistical evaluations?** | Yes |
| **Genotoxic Effects** | None |
| **Remarks for Results** | Results were not affected by the presence or absence of S9. |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce chromosomal aberrations in human lymphocytes. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Murthy, P.B.K., Ahmed, M.M., and Regu, K. (1991) Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes in vitro. Toxic In Vitro 5(4): 337-340. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Sister Chromatid Exchange |
| **System of Testing** | Non bacterial |
| **GLP** | Ambiguous |
| **Year** | 1991 |
| **Species/Strain** | Human lymphocyte |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat |
| **Doses/Concentration** | 0.1, 1.0, 10 mM with and without S9 |
| **Remarks for Test Conditions** | Lymphocytes were obtained from healthy donors (12 of each sex) and cultured at approximately 0.5-1.0E6 isolated cells. All cultures contained BrdU and metaphase preparations were stained with Hoechst-Giemsa stain. From each culture, a |

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| | minimum of 25 2nd-division cells were scored and the mean no. of SCEs/cell were compared with solvent controls. Solvent control was DMSO and positive control was MMC. |
| **Results** | Total no. of SCEs/cell (mean) for DMSO, DMSO+S9, MMC, 0.1 mM, 0.1 mM+S9, 1.0 mM, 1.0 mM+S9, 10 mM, and 10 mM+S9 was 7.90, 8.00, 21.46, 7.39, 8.25, 8.00, 8.10, 8.50, and 8.60, respectively. |
| **Appropriate statistical evaluations?** | Yes |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not affect the frequency of SCEs in human lymphocytes. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Murthy, P.B.K., Ahmed, M.M., and Regu, K. (1991) Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes in vitro. Toxic In Vitro 5(4): 337-340. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Galloway et al. (1985, 1987) |
| **Test Type** | Sister Chromatid Exchange |
| **System of Testing** | Non bacterial |
| **GLP** | Ambiguous |
| **Year** | 1989 |
| **Species/Strain** | Hamster/Chinese ovary cell |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat |
| **Doses/Concentration** | Trial 1: 0, 5, 16.7, or 50 ug/ml<br>Trial 2: 0, 2.5, 5, 10, or 25 ug/ml<br>Trial 3: 0, 16.7, 50, or 167 ug/ml |
| **Remarks for Test Conditions** | Positive controls used were mitomycin C (MMC) and cyclophosphamide (CP). Without S9, cultures were exposed to test substance for 25 hours. With S9, cultures were exposed for 2 hours. All cultures contained BrdU, which was added 2 hours after initial exposure, and cultures were exposed to BrdU until harvest. Typically, total incubation was 27.5-28 hours with Colcemid present 2-2.5 hours prior to harvest. Mitotic cells were |

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| | obtained and slides were prepared and examined by fluorescence microscopy after staining with Hoescht 33258 to evaluate cell cycle kinetics. For SCEs, cells were stained and scored. 50 cells were scored in trial 1 and 25 cells were scored in trials 2 and 3. |
| **Results** | SCE/cell for<br><br>Trial 1 (-S9) at 0, 5, 16.7, and 50 ug/ml: 7.72, 9.34. 8.06, and 7.94<br><br>Trial 2 (-S9) at 0, 2.5, 5, 10, and 25 ug/ml: 8.36, 7.78, 9.02, 9.75, and 10.04<br><br>Trial 3 (+S9) at 0, 16.7, 50, and 167 ug/ml: 8.42, 8.10, 8.74, and 8.48 |
| **Genotoxic Effects** | In trial 1, a less than 20% increase in SCEs was reported at the low dose only. In trial 2, none of the concentrations showed an increase over 20%, but the test was considered equivocal based on a positive trend. No effects were reported in the presence of S9 in trial 3. |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce SCE in Chinese hamster ovary cells. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. Part of NTP study program. |
| **References** | Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeiger E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Envir. Molecular Mut., 14(3), 165-187.<br><br>Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., and Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ Mutagen 7:1-51.<br><br>Galloway, S.M. Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S.,Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987)Chromosome aberration and sister chromatid exchange tests in vitro in Chinesehamster ovary cells: Results for 108 chemicals. Environ Molec Mutagen 10 (Suppl. 10):1-175. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Galloway *et al*. (1985, 1987) |

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| **Test Type** | Chromosomal Aberration assay |
| **System of Testing** | Non-bacterial |
| **GLP** | Ambiguous |
| **Year** | 1989 |
| **Species/Strain** | Hamster/Chinese ovary cell |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat |
| **Doses/Concentration** | Trial 1: 100, 150, or 200 ug/ml<br>Trial 2: 50, 125, or 250 ug/ml |
| **Remarks for Test Conditions** | Positive controls used were mitomycin C (MMC) and cyclophosphamide (CP). Without S9, cells were cultured with test article for 8 hours, then washed, then treated with Colcemid for 2-2.5 hours. With S9, cells were cultured with test article and S9 for 2 hours, washed, incubated for 8 hours then treated with Colcemid for 2-2.5 hours. Cells were harvested and slides prepared using Giemsa stain. 100-200 cells from each of the 3 highest scorable doses were analyzed and all aberrations were individually classified. Only the total percent cells with aberrations was considered in the statistical evaluation. |
| **Results** | Total percent cells with aberrations:<br>Trial 1 at 0, 100, 150, or 200 ug/ml: 4, 2, 2, or 4<br>Trial 2 at 0, 50, 125, or 250 ug/ml:4, 2, 1, or 4. |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce chromosomal aberrations in Chinese hamster ovary cells. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles. Part of NTP study program. |
| **References** | Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeiger E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Envir. Molecular Mut., 14(3), 165-187.<br>Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., and Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ Mutagen 7:1-51.<br>Galloway, S.M. Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S.,Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987)Chromosome aberration and sister chromatid exchange tests in vitro in Chinesehamster ovary cells: Results for 108 chemicals. Environ Molec Mutagen 10 |

(Suppl. 10):1-175.

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Ishidate and Odashima, 1977 |
| **Test Type** | Chromosomal Aberration assay |
| **System of Testing** | Non bacterial |
| **GLP** | No |
| **Year** | 1984 |
| **Species/Strain** | Hamster/Chinese fibroblast cell line |
| **Metabolic Activation** | Max. Concentration = 0.2 mg/ml |
| **Remarks for Test Conditions** | Cells were exposed to 3 different concentrations of the test substance for 24 or 48 hours after which colcemid was added 2 hours before harvesting. Cells were trypsinized, suspended in a hypotonic KCl solution (13 min at room temperature), centrifuged, fixed with acetic acid-methanol and applied to slides. Preparations were stained with Giemsa solution and 100 well-spread metaphases were microscopically observed. The incidence of polyploid cells and cells with structural chromosomal aberrations were counted. Controls consisted of solvent-treated or untreated cells. Test chemicals were considered positive if the incidence of aberrations was greater than 10%, equivocal if between 5.0 and 9.9%, and negative if less than 4.9%. For positive samples, the D20 (dose in mg/ml at which structural aberrations were detected in 20% of the metaphases observed) was calculated to assess the clastogenic potential. The frequency of cells with exchange-type aberrations per unit dose (mg/ml) was also calculated and expressed as “TR”. |
| **Results** | 0% polyploid<br>4.0% structural aberrations |
| **Cytotoxic concentration** | Not given |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce chromosomal aberrations in Chinese hamster fibroblasts. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., and Matsuoka, A. (1984). Primary mutagenicity |

screening of food additives currently used in Japan. Fd Chem Toxic 22(8):623-636.

Ishidate, M., Jr., and Odashima, S. (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro--A screening for chemical carcinogens. Mutat Res 48:337.

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Clive *et al*. (1979); Mitchell et al. (1988); Myhr and Caspary (1988) |
| **Test Type** | Forward mutation assay |
| **System of Testing** | Non-bacterial |
| **GLP** | Ambiguous |
| **Species/Strain** | Mouse/L5178Y lymphoma cell |
| **Metabolic Activation** | S9 from liver of Aroclor-induced male Sprague-Dawley rat |
| **Doses/Concentration** | + and -S9: 0, 12.5, 25, 50, 75, 100, 150, 200, and 300 ug/ml |
| **Remarks for Test Conditions** | Two trials (3 reps/concentration) were conducted for each: non-activated cultures and activated cultures. RPMI 1640 medium used for growth, expression and cloning. Ethanol used as vehicle control. |
| **Results** | Test substance cytoxic at 200 ug/ml and higher with or without S9.<br>Without S9,<br>Trial 1 at 0, 12.5, 25, 50, 100, and 150 ug/ml: 37, 36, 27, 38, 29, and 33 mutant cells/10E6 cells<br>Trial 2 at 0, 25, 50, 75, 100, and 150 ug/ml: 27, 30, 24, 19, 28, and 29 mutant cells/10E6 cells<br>With S9,<br>Trial 1 at 0, 25, 50, 75, 100, 150, and 200 ug/ml: 35, 42, 43, 45, 33, 33, and 35 mutant cells/10E6 cells<br>Trial 2 at 0, 25, 50, 75, 100, and 150 ug/ml: 48, 54, 58, 53, 64, and 37 mutant cells/10E6 cells |
| **Cytotoxic concentration** | 200 ug/ml |
| **Genotoxic Effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not increase mutation frequency in mouse lymphoma cells. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |

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| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. Part of NTP study program. |
| **References** | Myhr, B.C. and Caspary, W.J. (1991) Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the National Toxicology Program. Environ Mol Mutagen 18:51-83.<br><br>Clive, D., Johnson, K.D., Spectory, J.F.S., Batson, A.G., Brown, M.M.M. (1979) Validation and characterization of the L5178Y TK-/- mouse lymphoma mutagen assay system. Mutat Res 59:61-108.<br><br>Mitchen, A.D., Myhr, B.C., Rudd, C.J., Caspary, W.J., Dunkel, V.C. (1988) Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Methods used and chemicals evaluated. Environ Mol Mutagen 12(Suppl 13):1-18.<br><br>Myhr, B.C., Caspary, W.J. (1988) Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. Envrion Mol Mutagen 12(Suppl13):103-194. |

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| **Substance Name** | 4-*tert*-Butylcyclohexyl acetate |
| **CAS No.** | 32210-23-4 |
| **Method/guideline** | Ames Test |
| **Test Type** | Ames reverse mutation |
| **System of Testing** | Bacterial |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA98, TA 100 |
| **Metabolic Activation** | With and without |
| **Doses/Concentration** | 8-5000 ug/plate |
| **Remarks for Test Conditions** | - Metabolic activation system: Arochlor induced rat liver S9 mix<br><br>- Number of replicates: 2 Preincubation method<br><br>- Application: 8 to 5000 microg/plate<br><br>- Positive and negative control groups and treatment: positive controls:<br><br>TA 98, TA 158: 2.5 ug Nitrofluorene/plate<br><br>TA 100, TA 1535: 2.5 ug Sodium-azid/plate<br><br>TA1537: 50 ug aminoacridine/plate<br><br>Negative control: solvent: Dimethylsulfoxide |
| **Results** | Negative |

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| **Cytotoxic concentration** | 200 and 400 micrograms/plate |
| **Genotoxic Effects** | GENOTOXIC EFFECTS:<br>- With metabolic activation: not genotoxic<br>- Without metabolic activation: not genotoxic |
| **Remarks for Results** | Toxic effects were observed at concentrations 200 ug/plate or higher. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Degussa AG (1989) Unpublished report. Report No, 89-350-DKM. |

### 4.2.2 *In vivo* Genotoxicity

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Chromosomal aberration |
| **Test Type** | Cytogenetic assay-Acute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Rat/Albino |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5, or 145 mg/kg bw; test 2: 500 or 3000 mg/kg bw |
| **Exposure Period** | 6, 24 or 48 hours |
| **Remarks for Test Conditions** | Groups of rats were gavaged with 1.45, 14.5 or 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 500 or 3000 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2) and groups of rats were killed at 6, 24 and 48 hours. 4 hours after administration and 2 hours prior to termination, rats were intraperitoneally injected with 4 mg colcemid/kg bw. Bone |

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| | marrow was removed and slides were prepared and analyzed. |
| **Genotoxic effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce chromosomal aberrations. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Chromosomal aberration |
| **Test Type** | Cytogenetic assay-Subacute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Rat/Albino |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5, or 145 mg/kg bw; test 2: 1150 mg/kg bw |
| **Exposure Period** | Five doses 24 hours apart |
| **Remarks for Test Conditions** | Groups of rats were gavaged with 1.45, 14.5 or 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 1150 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2) for 5 consecutive doses, 24 hours apart and were killed 6 hours after last dose. 4 hours after administration and 2 hours prior to termination, rats were intraperitoneally injected with 4 mg colcemid/kg bw. Bone marrow was removed and slides were prepared and analyzed. |
| **Genotoxic effects** | None |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol did not induce chromosomal aberrations. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245- |

444 (FDA 71-268).

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Micronucleus test |
| **GLP** | Ambiguous |
| **Year** | 1993 |
| **Species/Strain** | Mouse/B6C3F1 |
| **Sex** | Male |
| **Route of Administration** | Intraperitoneal |
| **Doses/Concentration** | 0, 250, 500, and 1,000 mg/kg bw |
| **Exposure Period** | 3 daily exposures |
| **Remarks for Test Conditions** | Groups of 5-6 mice were intraperitoneally injected on 3 consecutive days with 1X, 0.5X and 0.25X of the test chemical. A positive control and solvent control were also used. 24 hours after the last treatment, mice were killed, bone marrow removed and slides were prepared. For each mouse, the number of MN-PCE in 2,000 PCE and the percent PCE in 200 erythrocytes were determined. |
| **Effect on mitotic index or PCE/NCE ratio by dose level and sex** | 0 mg/kg bw: survival=5/5 mice; MN-PCE/1000=2.90; %PCE=54.4<br>250 mg/kg bw: survival=5/5 mice; MN-PCE/1000=3.60; %PCE=64.2<br>500 mg/kg bw: survival=5/5 mice; MN-PCE/1000=2.20; %PCE=56.7<br>1000 mg/kg bw: survival=3/6 mice; MN-PCE/1000=3.67; %PCE=51.8 |
| **Genotoxic effects** | None |
| **Appropriate statistical evaluations** | Yes |
| **Conclusion Remarks** | 2-Isopropyl-5-methylcyclohexanol was negative in the micronucleus test. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. Part of NTP study program. |
| **References** | Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993) Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ Mol |

Mutagen 21:160-179.

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Host-mediated-Acute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Mouse/ICR |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5 and 145 mg/kg bw; Test 2: 500 and 5000 mg/kg bw |
| **Exposure Period** | Single exposure |
| **Remarks for Test Conditions** | Indicator organisms were *Salmonella typhimurium* strains G46 and TA1530, and *Saccharomyces cervisiae* D3.<br>Groups of mice were given 1.45, 14.5 and 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 500 or 5000 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2) by gavage followed by intraperitoneal injection of 2 ml indicator organism. Three hours later, mice were killed and intraperitoneally injected with 2 ml of sterile saline. As much fluid as possible was removed from the peritoneal cavity and dilutions were made from each exudate. Dilutions were plated and incubated for 18-40 hours. Further dilutions were made, plated and incubated at 30 °C for 40 hours after which bacterial scoring was conducted for calculation of mutation frequency and recombinant frequency. |
| **Genotoxic effects** | Only at 5000 mg/kg bw in *Salmonella* TA1530. |
| **Remarks for Results** | In vitro tests using same organisms were all negative. |
| **Conclusion Remarks** | No significant increase in mutant and recombinant frequency at any dose in *Salmonella* G46 and *Saccharomyces* D3. At the highest dose tested in *Salmonella* TA1530 a significant increase in mutant frequency was reported. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Host-mediated-Subacute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Mouse/ICR |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5 and 145 mg/kg bw; Test 2:1150 mg/kg bw |
| **Exposure Period** | Five doses 24 hours apart |
| **Remarks for Test Conditions** | Indicator organisms were *Salmonella typhimurium* strains G46 and TA1530, and *Saccharomyces cervisiae* D3.<br><br>Groups of mice were given 1.45, 14.5 and 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 1150 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2) by gavage for 5 consecutive doses, 24 hours apart. Thirty minutes after the last dose, mice were given an intraperitoneal injection of 2 ml indicator organism. Three hours later, mice were killed and intraperitoneally injected with 2 ml of sterile saline. As much fluid as possible was removed from the peritoneal cavity and dilutions were made from each exudate. Dilutions were plated and incubated for 18-40 hours. Further dilutions were made, plated and incubated at 30 °C for 40 hours after which bacterial scoring was conducted for calculation of mutation frequency and recombinant frequency. |
| **Genotoxic effects** | Elevated recombinant frequency in *Saccharomyces* D3 |
| **Conclusion Remarks** | No significant increase in mutant and recombinant frequency at any dose in *Salmonella* G46 and TA1530, but in *Saccharomyces* D3 an elevation of recombinant frequency was reported. In vitro tests using same organisms were all negative. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |


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| **Substance Name** | 4-*tert*-Butylcyclohexanol |

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| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Dominant lethal assay-Acute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Rat/Random bred |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5, or 145 mg/kg bw; test 2: 500 or 3000 mg/kg bw |
| **Exposure Period** | Single dose |
| **Remarks for Results** | Groups of male rats were gavaged with 1.45, 14.5 or 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 500 or 3000 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2). Male rats were mated with 2 female rats per week for 8 weeks. Fourteen (14) days after mating, females were killed and the uterus was examined for early deaths, late fetal deaths and total implantations. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

## 4.3 Repeated Dose Toxicity

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Method/guideline** | OECD Guideline 407 |
| **GLP** | Yes |
| **Year** | 1998 |

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| **Species/strain** | Rat/Wistar |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Gavage |
| **Doses/concentration Levels** | 50, 150, 300 mg/kg bw/day |
| **Exposure Period** | 28 days |
| **Frequency of Treatment** | Once daily |
| **Control Group** | Yes, concurrent vehicle |
| **Post exposure observation period** | 14 days |
| **Remarks for Test Conditions** | TEST ORGANISMS<br>- Age: 6 to 8 weeks<br>- Weight at study initiation: 183-188.9 g variation less than 20%<br>- Number of animals: 30 male, 30 female<br>ADMINISTRATION / EXPOSURE<br>- Duration of test/exposure: 28 day<br>- Type of exposure: gavage<br>- Post exposure period: 14 days<br>- Vehicle: corn oil<br>- Concentration in vehicle: 10, 30, 60 mg/ml<br>- Total volume applied: 5 ml/kg<br>- Doses: 50, 150, 300 mg/kg per day<br>CLINICAL OBSERVATIONS AND FREQUENCY:<br>- Clinical signs: general health: twice daily, general clinical observations: once daily.<br>A detailed FOB in home, cage and open field was conducted once per week. During the final week of the dosing period all animals were subject to an extended FOB, extended open field observation test, sensory reactivity to different stimuli, motor activity assessment, landing foot splay, grip strength, rearing behaviour.<br>- Body weight: At allocation of the groups, prior to first dose, weekly.<br>- Food consumption: weekly<br>- Water consumption: daily<br>- Haematology, at the end of the treatment period/recovery period:<br>RBC, WBC, Platelet count, Haemoglobin, Hematocrit, MCV, Mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration. Differential WBC, coagulation.<br>- Biochemistry: at the end of the treatment period/recovery |

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| | period: sodium, potassium, calcium, AST, ALT, AP, Glucose, Triglycerides, cholesterol, total bilirubin, BUN, Creatinine, total protein, albumin.<br><br>- Urinalysis: after 6 hours in metabolic cages before termination, Volume, pH, specific gravity, colour, protein, glucose, ketones, urobilinogen, blood, sediment analysis for leukocytes, erythrocytes, bacteria, epithelial cells squamous and renal, oxalate crystals, triple phosphate crystals, carbonate, granular cylinder, urate crystals.<br><br>ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):<br><br>- Macroscopic:<br><br>Organ weights of following organs were determined: liver, kidney, adrenals, spleen, heart, thymus, brain, male gonads and epididymis.<br><br>- Microscopic:<br><br>The following organs and all gross lesions were preserved in formalin for all dose groups. In high dose and controls all organs were examined histopathologically and selected organs of the other dose groups were also examined.<br><br>Organs: Adrenals, aorta, anus, brain, all parts of the intestinal tract, epididymides, eyes, exorbital lacrimal glands, heart, kidneys, larynx, liver, lungs, lymph nodes (skin, cervical, mesenteric), mammary gland, muscle, ovaries, oesophagus, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, stomach, sternum, testes, thymus, thyrois, parathyrois, Tongue, Trachea, unrinary bladder, uterus, vagina.<br><br>STATISTICAL METHODS:<br><br>For FOB: Kruskal Wallis non parametric analysis of variance, in case of significance: pairwise comparison with Wilcoxon, Mann, Whitney U-Test.<br><br>Other data: ANOVA or Kruskal Wallis test if heterogenous |
| **NOAEL(NOEL)** | 50 mg/kg bw |
| **LOAEL(LOEL)** | 150 mg/kg/bw |
| **Toxic Response/effects by Dose Level** | Mortality: 2 animals of the high-dose group died due to an application failure and were replaced by two recovery animals. Clinical symptoms: 15 min after test substance administration high and medium dose group: convulsions, squatting position, straub tail (one female animal of the high-dose group) and vocalization. Some animals were walking on tiptoes. The symptoms disappeared within a few hours to 1 day. No clinical abnormalities were observed in the controls and recovery animals. FOB: After the first and second day of the treatment period no abnormalities were observed. After 2, 3 and 4 weeks of treatment clinical symptoms were observed in individual animals predominantly in the high-dose group. The effects included ataxia, fasciculations, padding movements, defense against touching, aggressiveness, hunchback/squatting |

position, reduced respiration, hyperactivity, straub tail, slight convulsions. In the recovery period (week 5 and 6) no significant treatment-related clinical signs were observed in all dose groups. Extended FOB week 4: Reactivity to standard stimuli was normal in the control, low and medium dose animals. Individual animals of the high-dose group showed minimal to high sensitivity of pain. One male showed catalepsy. Rearing, landing foot splay and grip strength was normal in the control, low, medium and high-dose groups and in the recovery group females. High-dose male recovery group animals showed a statistically significant increase of group mean values of landing-foot-splay and rearing and a decrease of group mean values of grip strength compared to recovery controls, but these differences were minor and did not show a consistent pattern in the individual animals. The effects were also not seen in the high-dose group males that were not allocated to the recovery group at the same examination time. Therefore the findings were considered of minor toxicological importance. No statistically significant increase in motor activity was observed in all dose groups comparing group mean values to that of controls. A statistically non-significant increase was observed in high-dose animals, in particular females. Body weights: A slight decrease in body weight and body weight changes was observed in the high-dose males (statistically significant only for high-dose recovery group males in week 4. The females of the high-dose recovery group showed an increase of group mean body weight change at the beginning of the study. In the recovery period no statistically significant differences in body weights and body weight gains were observed. Food intake: A slight reduction was seen in treated males and an increase in treated females when compared to controls. During the recovery period the food consumption of the treated animals was increased compared to controls. Water consumption was not different between treated and control groups. Alterations in clinical chemistry, urinalysis and hematology parameters were minor and within the normal range of the historical data. All differences observed were considered of minor toxicological importance. Organ weights: At the end of the treatment period male high-dose group animals showed a statistically significant increase in relative adrenal weight when compared with controls. At the end of the recovery period the treated males revealed a statistically significant increase in relative epididymis weight compared to the controls. Histopathology: Treatment-related findings were restricted to an increased number of male animals of the high-dose group with eosinophilic hyaline droplets in the epithelial cell cytoplasm of the proximal tubules (5 treated compared to 1 control). This could be indicative of the alpha-2 microglobulin nephropathy syndrome in male rats that is a rat-specific effect. The deaths were not treatment related.

**Remarks for Results**

Based on the clinical signs a NOAEL of 50 mg/kg bw and a LOAEL of 150 mg/kg bw were derived. *Alpha*-2-microglobulin effects in males were reported at the highest dose levels.

**Conclusion Remarks**

The main effects of the test substance are clinical signs of neurotoxicity ant the high and mid dose. No histopathological changes that are considered relevant for humans were

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| | observed. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Guideline study. |
| **References** | Degussa AG (1999) Unpublished report. Report No. 98-0184-DGT. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data for mixture of structurally related alkyl-substituted cyclohexanols and cyclohexanones:<br>1) 46.8% (1a, 2beta, 5a)-2-isopropy-5-methylcyclohexanol<br>2) 3.97% (1a, 2a, 5a)-2-isopropy-5-methylcyclohexanol<br>3) 0.86% (1 beta, 2beta, 5a)-2-isopropy-5-methylcyclohexanol<br>4) 21.81% (2beta, 5a)-2-isopropy-5-methylcyclohexanone<br>5) 3.07% (2beta, 5beta)-2-isopropy-5-methylcyclohexanone<br>6) 5.11% (1a, 2beta, 5a)-2-isopropy-5-methylcyclohexyl acetate<br>7) 1.55% (1beta, 2beta, 5 beta)-2-isopropy-5-methylcyclohexyl acetate.<br>The other constituents accounting for approximately 10% of the oil include aliphatic terpene hydrocarbons (*e.g., alpha*-pinene) and ethers (eucalyptol) (Vollmuth, 1989). |
| **Method/guideline** | 28-Day Oral Toxicity Study |
| **GLP** | Yes |
| **Year** | 1990 |
| **Species/strain** | Rat/Sprague Dawley |
| **Sex** | Male and Female |
| **Route of Administration** | Oral Gavage |
| **Doses/concentration Levels** | 0, 100, 200, or 400 mg/kg bw |
| **Exposure Period** | 29 or 30 |
| **Frequency of Treatment** | Once daily |
| **Control Group** | Yes, vehicle only |
| **Remarks for Test Conditions** | Groups (10/sex/group) of male and female Sprague-Dawley rats were given daily dose levels of 0, 100, 200, or 400 mg/kg bw of a mixture of alkyl-substituted cyclohexanol by gavage in corn oil (10 ml/kg) daily for 29 or 30 days. Clinical signs were monitored twice weekly and body weights and food consumption were measured weekly. At the initiation of the study, 10 animals were randomly selected from the pool of |

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| | animals not selected fro the study. They were fasted overnight and blood samples were drawn and analyzed for baseline clinical chemistry and hematology parameters. Prior to termination, animals were injected with ketamine and blood samples were drawn for clinical chemistry and hematology. At necropsy, organ weights (brain, spleen, liver, heart, kidneys, testes with epididymides, adrenals, ovaries, and pituitary) were measured, and tissues (26) were preserved in 10% formalin. All tissues from the control and high-dose groups and tissues from the heart, liver, kidneys, and gross lesions from the low- and mid-dose group were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically. |
| **NOAEL(NOEL)** | Less than 100 mg/kg bw per day for males and 400 mg/kg bw per day for females |
| **LOAEL(LOEL)** | 100 mg/kg bw per day (based on appearance of alpha-2-microglobulin effect in males) |
| **Actual dose received by dose level and sex** | 0, 100, 200, or 400 mg/kg bw |
| **Toxic Response/effects by Dose Level** | All animals survived to study termination with high dose males showing increased incidence of urine staining during clinical observations. Except for a non-statistically significant decrease in mean body weigh in high-dose males, there were no statistically significant differences in body weight or food consumption between treated and control groups. A significant decrease in serum glucose levels was reported in the mid- and high-dose males that the authors, in part, attribute to change in nutritional status as revealed by a decreased body weights in the high-dose group. A treatment-related increase in alkaline phosphatase was reported in high-dose males.<br><br>Measurement of body weight, food consumption, hematology and clinical chemistry parameters revealed no significant changes between test and control female rats. There were statistically significant increases in relative kidney weights in high-dose males. Histopathological findings revealed renal tubule protein droplets in all groups of treated male rats. The authors considered these findings related to the lysosomal handling of alpa-2-micro-globulin, a protein specific to the male Sprague-Dawley rat. Absolute and relative liver weights in high-dose females also were significantly increased but these changes were not confirmed by histopathological examination. |
| **Appropriate statistical evaluations?** | Dunnett's Control versus Treatment Comparison. |
| **Remarks for Results** | Based exclusively on the renal pathology (*alpha*-2-microglobulin effect) reported in all dosed groups of male rats, the authors concluded that the no observable adverse effect level (NOAEL) for the mixture is less than 100 mg/kg bw per day in male rats and 400 mg/kg bw per day in female rats |
| **Conclusion Remarks** | The NOAEL is less than 100 mg/kg bw in male Sprague-Dawley rats and 400 mg/kg bw per day for female rats. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restriction. |

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| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Serota D. G. (1990) 28-Day oral toxicity study in rats: B100. HLA Study No. 642-477. Private Communication to FEMA. Unpublished Report. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | 90-Day Toxicity Study |
| **GLP** | Yes |
| **Year** | 1979 |
| **Species/strain** | Mousr/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Diet |
| **Doses/concentration Levels** | 0, 930, 1870, 3750, 7500, or 15000 ppm (~0, 140, 281, 563, 1125 or 2250 mg dl-2-isopropyl-5-methylcyclohexanol/kg bw/day) |
| **Exposure Period** | 13 weeks |
| **Frequency of Treatment** | Daily |
| **Control Group** | Basal diet with 2% corn oil |
| **Remarks for Test Conditions** | Groups of 10 male and 10 female B6C3F1 mice were maintained on diets containing dl-2-isopropyl-5-methylcyclohexanol at dietary concentrations of 0, 930, 1870, 3750, 7500, or 15000 ppm for 13 weeks. Dietary concentrations were calculated to provide average daily intake levels of 0, 140, 281, 563, 1125 or 2250 mg dl-2-isopropyl-5-methylcyclohexanol/kg bw, respectively. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from the control animals, the 2250 mg/kg bw/day group, and selected tissues from the 1125 mg/kg bw/day group. |
| **NOAEL(NOEL)** | 1125 mg/kg bw/day |
| **LOAEL(LOEL)** | 563 mg/kg bw/day |
| **Toxic Response/effects by Dose Level** | Six mice (sex not specified) died during the study but the deaths could not be attributed to compound administration. Final mean body weights of the male mice and female mice were not statistically different from those of the controls except for the high-dose female group which showed statistically significant decreased body weights. A slight increase in the incidence of perivascular lymphoid hyperplasia and interstitial nephritis was |

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| | reported in the female mice given the two highest dose levels. No adverse effects were reported for male or female mice administered 140, 281 or 563 mg dl-2-isopropy-5-methylcyclohexanol/kg bw/day. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **References** | National Cancer Institute, NCI (1979) Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education and Welfare. National Technical Report Series No. 98. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | 90-Day Toxicity Study |
| **GLP** | No |
| **Year** | 1979 |
| **Species/strain** | Rat/F344 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Diet |
| **Doses/concentration Levels** | 0, 930, 1870, 3750, 7500, or 15000 ppm (~0, 93, 187, 375, 750 or 1500 mg dl-2-isopropy-5-methylcyclohexanol/kg bw/day) |
| **Exposure Period** | 13 weeks |
| **Frequency of Treatment** | Daily |
| **Control Group** | Basal diet with 2% corn oil |
| **Remarks for Test Conditions** | Groups of 10 female and 10 male Fischer 344 rats per group were maintained on diets containing dl-menthol at concentrations of 0, 930, 1870, 3750, 7500, or 15000 ppm for 13 weeks. Dietary concentrations were calculated to provide corresponding average daily intake levels of 0, 93, 187, 375, 750 or 1500 mg dl-2-isopropy-5-methylcyclohexanol/kg bw, respectively. Necropsies were performed on all animals at the end of the study. Histopathological examination was performed on tissues from the control animals, the highest dose group, and selected tissues from the second highest dose group. |
| **NOAEL(NOEL)** | 750 mg/kg bw/day |
| **LOAEL(LOEL)** | 1500 mg/kg bw/day |
| **Toxic Response/effects by Dose Level** | Final mean body weights of the male and female rats at all dose levels were similar to those of the controls. A slight increase in the incidence of interstitial nephritis was observed in high-dose |

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| | male rats. No adverse effects were reported for male or female rats administered 93, 187, 375, or 750 mg dl-2-isopropy-5-methylcyclohexanol/kg bw/day. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **References** | National Cancer Institute, NCI (1979) Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education and Welfare. National Technical Report Series No. 98. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Carcinogenicity Study |
| **GLP** | No |
| **Year** | 1979 |
| **Species/strain** | Rat/F344 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Diet |
| **Doses/concentration Levels** | 0, 3750, or 7500 ppm (approximately 0, 187 or 375 mg/kg bw, respectively) |
| **Exposure Period** | 103 weeks |
| **Frequency of Treatment** | Daily |
| **Control Group** | Basal diet with 2% corn oil |
| **Post exposure observation period** | 2 weeks |
| **Remarks for Test Conditions** | Groups of 50 Fischer 344 rats of each sex were administered 0, 3750 or 7500 ppm dl-2-isopropy-5-methylcyclohexanol in their feed daily for 103 weeks. Dietary concentrations were calculated to provide corresponding average daily intake levels of approximately 0, 187 or 375 mg/kg bw, respectively. Animals were housed five per cage until week 48 when the male rats were divided into groups of two to three per cage. The animals were observed twice daily for signs of toxicity. Body weight and food consumption were recorded every two weeks for the first twelve weeks, and once a month thereafter. Necropsies and histological examinations were performed on all animals at the termination of the study and on those found dead during the study. |
| **NOAEL(NOEL)** | 375 mg/kg bw/day |

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| **Toxic Response/effects by Dose Level** | The mean body weights of the male and female rats administered 187 or 375 mg/kg dl-menthol were slightly lower when compared to the controls. Survival of the high- and low-dose groups of male (controls, 31/50; low-dose, 33/50; high-dose, 34/50) and female (controls, 36/50; low-dose, 35/50; high-dose, 38/50) rats was similar to the control animals. Chronic inflammation of the kidney observed in the dosed older males was not considered by the authors to be related to the administration of dl-2-isopropy-5-methylcyclohexanol since the effect is commonly observed in aged male Fischer 344 rats. There was no increase in the incidence of neoplasms of dosed females compared to that of control animals. In the low-dose (10/49) and high-dose (7/49) female groups, fibroadenomas of the mammary glands occurred at a lower incidence than in the control group (20/50). Alveolar/bronchiolar adenomas or carcinomas were reported only for the female control rats. Under the conditions of this study, it was concluded that dl-2-isopropy-5-methylcyclohexanol was neither carcinogenic nor toxic for either sex of Fischer 344 rats at dose levels of 187 or 375 mg dl-2-isopropy-5-methylcyclohexanol /kg bw. |
| **Appropriate statistical evaluations?** | Yes |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **References** | National Cancer Institute, NCI (1979) Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education and Welfare. National Technical Report Series No. 98. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Test Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Method/guideline** | Carcinogenicity Study |
| **GLP** | No |
| **Year** | 1979 |
| **Species/strain** | Mouse/B6C3F1 |
| **Sex** | Male and Female |
| **Route of Administration** | Oral-Diet |
| **Doses/concentration Levels** | 0, 2,000 or 4,000 ppm (approximately 0, 300 or 600 mg/kg bw, respectively) |
| **Exposure Period** | 103 weeks |
| **Frequency of Treatment** | Daily |

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| **Control Group** | Basal diet with 2% corn oil |
| **Post exposure observation period** | 1 week |
| **Remarks for Test Conditions** | A carcinogenicity study was conducted in which groups of 50 B6C3F1 mice of each sex were administered 0, 2,000 or 4,000 ppm dl-2-isopropy-5-methylcyclohexanol in their feed daily for 103 weeks. Dietary concentrations were calculated to provide corresponding average daily intake levels of 0, 300 or 600 mg/kg bw, respectively. Animals were housed five per cage and were observed twice daily for signs of toxicity. Body weights and food consumption were recorded every two weeks for the first twelve weeks, and once a month thereafter. Necropsies and histological examinations were performed on all animals at the termination of the study and on those found dead during the study. |
| **NOAEL(NOEL)** | 300 mg/kg bw/day |
| **LOAEL(LOEL)** | 600 mg/kg bw/day |
| **Toxic Response/effects by Dose Level** | The mean body weights of the male and female mice administered 300 or 600 mg dl-2-isopropy-5-methylcyclohexanol/kg bw were slightly lower when compared to the controls. Survival of the high- and low-dose groups of male mice was similar to the vehicle control animals (controls, 32/50; low-dose, 32/50; high-dose, 35/50). Survival of the high-dose group of female mice was significantly less than that of the control animals (controls, 36/50; high-dose, 45/50). However, decreased survival was not accompanied by any evidence of toxicity in the high-dose group. Survival of the low-dose female mice was similar to the control animals (controls, 36/50; low-dose, 40/50). An increase in the incidence of hepatocellular carcinomas was observed in high-dose male mice (controls, 8/47; low-dose, 8/49; high-dose, 14/48), but was not statistically different from that observed historically in mice of that age and strain [Haseman *et al.,* 1986]. A low incidence of alveolar/bronchiolar adenomas of the lung was observed in both the low- and high-dose females but was not statistically different from the incidence of this neoplasm in historical control groups. Under the conditions of this study, it was concluded that dl-2-isopropy-5-methylcyclohexanol was not carcinogenic and did not produce any organ-specific toxicity for either sex of B6C3F1 mice at dose levels of 300 or 600 mg/kg bw. |
| **Appropriate statistical evaluations?** | Yes |
| **Remarks for results** | NOAEL based on decreased survival in female mice. |
| **Data Qualities Reliabilities** | Reliability code 1. Reliable without restriction. |
| **Remarks for Data Reliability** | Code 1. Comparable to guideline study. |
| **References** | National Cancer Institute, NCI (1979) Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education and Welfare. National Technical Report Series No. 98. |

Haseman J.K., Winbush J.S. and O'Donnell M.W. (1986) Use of dual control groups to estimate false positive rates in laboratory animal carcinogenicity studies. Fundamental and Applied Toxicology 7, 573-584.

## 4.4 Reproductive Toxicity

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data for mixture of structurally related alkyl-substituted cyclohexanols and cyclohexanones:<br>1) 46.8% (1*alpha*, 2 *beta*, 5 *alpha*)-2-isopropy-5-methylcyclohexanol<br>2) 3.97% (1 *alpha*, 2 *alpha*, 5 *alpha*)-2-isopropy-5-methylcyclohexanol<br>3) 0.86% (1 *beta*, 2 *beta,* 5 *alpha*)-2-isopropy-5-methylcyclohexanol<br>4) 21.81% (2 *beta*, 5 *alpha*)-2-isopropy-5-methylcyclohexanone<br>5) 3.07% (2 *beta*, 5 *beta*)-2-isopropy-5-methylcyclohexanone<br>6) 5.11% (1 *alpha*, 2 *beta*, 5 *alpha*)-2-isopropy-5-methylcyclohexyl acetate<br>7) 1.55% (1 *beta*, 2 *beta*, 5 *beta*)-2-isopropy-5-methylcyclohexyl acetate.<br>The other constituents accounting for approximately 10% of the oil include aliphatic terpene hydrocarbons (e.g*., alpha*-pinene) and ethers (eucalyptol) (Vollmuth, 1989). |
| **Method/Guideline** | *in vivo* Reproductive and Developmental Toxicity Screening Test |
| **GLP** | Yes |
| **Year** | 1989 |
| **Species/Strain** | Rat/Sprague Dawley |
| **Sex** | Female |
| **Route of Administration** | Oral-Gavage |
| **Duration of Test** | 39 days |
| **Doses/Concentration** | 0, 150, 750, or 1,500 mg/kg bw |

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| **Premating Exposure period for females** | 7 days |
| **Control Group and Treatment** | Yes, vehicle only (corn oil) |
| **Frequency of Treatment** | Daily |
| **Remarks for Test Conditions** | Groups of ten female rats were orally administered an oil containing a mixture of alkyl-substituted cyclohexanol derivatives via gavage at dose levels of 0, 150, 750 or 1500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weights, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on Day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 post partum, any pups dying during the lactation period were necropsied. |
| **NOAEL(NOEL)** | 150 mg/kg bw |
| **Actual dose received by dose level and sex** | 0, 150, 750, or 1,500 mg/kg bw |
| **Appropriate statistical evaluations** | Yes |
| **Parental data and F1 as Appropriate** | Deaths or moribund sacrifice were reported in 2/10 females at 750 mg/kg bw per day and 5/10 females at 1,500 mg/kg bw per day. Additional clinical observations included decreased motor activity, ataxia, dyspnea, rales, chromorrhinorrhea, un-groomed coat and thin appearance at the 750 and 1500mg/kg bw per day dose levels. Urine stained fur and excess salivation were observed at all dose levels. Significant (P < or = 0.05) decreases in body weight and food consumption were reported during the pre-mating period in the 750 and 1500 mg/kg bw per day groups compared to those for control group. A non-statistically significant decrease in maternal body weight gain was reported in the 750 mg/kg bw per day group compared to the control group. The single dam that delivered a litter in the high-dose group also showed less weight gain.<br><br>Absolute and relative feed consumption were comparable between the low-, mid, and control groups.<br><br>On day 1 of lactation, the average body weight of dams in the mid-dose group and the single dam in the high-dose group was significantly (P < or =0.01) less than in the control group. During lactation, dams in the mid-dose group gained weight while the weight gain in the single dam in the high-dose group were comparable to that for the control group. Compared to control animals, feed consumption in the mid- and high-dose group decreased significantly (P< or =0.01) during premating but was increased significantly (P< or =0.01 to 0.05) during lactation. Of the rats surviving the cohabitation period 4 of 5 became pregnant at the highest dose level (1500 mg/kg bw per day). |

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| | Live litters were reported for 9/19, 8/10, 5/6, and 1/4 pregnant females in the control, 150, 750, and 1500 mg/kg bw per day groups, respectively. Increased number of dams with stillborn pups, stillborn pups, and late resorptions in utero were reported in the 750 mg/kg bw per day group.<br><br>At 1500 mg/kg bw per day, 2 rats had only resorptions in utero when found dead on gestation day 23 and one rat possessed only empty implantation sites in utero on day 25 of presumed gestation. |
| **Offspring toxicity F1 and F2** | On day 1 postparturition, litters of dams in the 750 and 1500 mg/kg bw per day groups showed non-statistically significant decreases in pup weight which by day 4 were comparable to controls in the mid-dose group, but less than the control value in the high dose group. On day 4 postparturition, significant (P< or = 0.01) increases in pup mortality were reported in the mid- and high-dose groups compared to controls. However, even at the highest dose level, there was no evidence of an effect of the test article on implantation, duration of gestation, pup sex ratio, or gross morphology of pups. |
| **Conclusion remarks** | Authors concluded that the maternal NOAEL for reproductive effects was 150 mg/kg bw per day and the offspring NOAEL for developmental effects is higher than 150 mg/kg bw per day, but less than 750 mg/kg bw per day |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Comparable to guideline study with acceptable restrictions. |
| **References** | Hoberman A. M. (1989) Reproductive and developmental toxicity screening of B100 administered orally *via* gavage to Crl:CD(SD)Br female rats. Argus Research Laboratories, Inc. Protocol 412-015. Private Communication to FEMA. Unpublished Report. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Dominant lethal assay-Acute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Rat/Random bred |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |

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| **Frequency of treatment** | Single dose |
| **Doses/Concentration** | Test 1: 1.45, 14.5, or 145 mg/kg bw; test 2: 500 or 3000 mg/kg bw |
| **Control Group and Treatment** | Saline |
| **Remarks for Test Conditions** | Groups of male rats were gavaged with 1.45, 14.5 or 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 500 or 3000 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2). Male rats were mated with 2 female rats per week for 8 weeks. 14 days after mating, females were killed and the uterus was examined for early deaths, late fetal deaths and total implantations. |
| **Conclusion remarks** | No effect on early deaths, late fetal deaths and total implantations was reported when 2-isopropyl-5-methylcyclohexanol was administered to male rats prior to mating. |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Dominant lethal assay- Subacute study |
| **GLP** | No |
| **Year** | 1975 |
| **Species/Strain** | Rat/Random bred |
| **Sex** | Male |
| **Route of Administration** | Oral-Gavage |
| **Doses/Concentration** | Test 1: 1.45, 14.5, or 145 mg/kg bw; test 2: 1150 mg/kg bw |
| **Control Group and Treatment** | Saline |
| **Frequency of Treatment** | Five doses 24 hours apart |
| **Remarks for Test Conditions** | Groups of rats were gavaged with 1.45, 14.5 or 145 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 1) or 1150 mg 2-isopropyl-5-methylcyclohexanol/kg bw (test 2) for 5 consecutive doses, 24 hours apart. After the last dose, male rats were mated with 2 female rats per week for 7 weeks. 14 days after mating, females were killed and the uterus was examined for |

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| | early deaths, late fetal deaths and total implantations. |
| **Conclusion remarks** | No effect on early deaths, late fetal deaths and total implantations was reported when 2-isopropyl-5-methylcyclohexanol was administered to male rats prior to mating. |
| **Data Reliabilities Qualities** | Reliability code 2. Reliable with restriction. |
| **Remarks for Data Reliability** | Code 2. Basic data given: comparable to guidelines/standards. |
| **References** | Food and Drug Administration (FDA) (1975) Mutagenic evaluation of compound FDA 71-57, menthol. NTIS PB-245-444 (FDA 71-268). |

## 4.5 Developmental/Teratogenicity Toxicity

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Teratology study |
| **GLP** | Pre GLP |
| **Year** | 1973 |
| **Species/strain** | Mouse/CD-1 outbred |
| **Sex** | Female |
| **Route of Administration** | Gavage |
| **Duration of Test** | 10 days |
| **Doses/concentration Levels** | 0(negative control), 0, 1.85, 8.59, 39.9, 185 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 15 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female CD-1 outbred mice were gang-housed in plastic disposable cages in |

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| | a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (22-23/group) of pregnant females were given 0, 1.85, 8.59, 39.9, 185 mg/kg bw of the test material (FDA 71-57) by gavage in corn oil. A positive control group received 150-mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, number of resorptions, % of live and % partial live resorptions, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL) maternal toxicity** | 185 mg/kg bw/day |
| **NOAEL (NOEL) developmental toxicity** | 185 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 1.85, 8.59, 39.9, 185 mg/kg bw of the test material (FDA 71-57) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female mice. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. The number of live litters, average implant sites per dam were similar for both test and control groups. The % partial resorptions and % complete resorption were increased for the 1.85 and 8.59 mg/kg bw groups, but higher dose levels exhibited lower resorption rates compared to the control groups. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses was similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and negative control groups. There was evidence of incomplete ossification in the positive control group. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and negative control animals. Visceral examination failed to reveal any evidence of soft tissue abnormalities at any dose level. |

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| **Conclusion remarks** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 185 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restrictions. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973a) Teratologic evaluation of FDA 71-57 in mice. Contract No. FDA 71-260. Unpublished Report. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Teratology study |
| **GLP** | Pre GLP |
| **Year** | 1973 |
| **Species/strain** | Rat/female Wistar |
| **Sex** | Female |
| **Route of Administration** | Gavage |
| **Duration of Test** | 10 days |
| **Doses/concentration Levels** | 0(control), 2.18, 10.15, 47.05, 218 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 15 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female rats were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (22-25/group) of pregnant females were given 0, 2.18, 10.15, 47.05, 218 mg/kg bw of the test material (FDA 71-57) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all |

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| | dams were subjected to Caesarian section and the number of implantation sites, number of resorptions, % of live and % partial live resorptions, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL) maternal toxicity** | 218 mg/kg bw/day |
| **NOAEL (NOEL) developmental toxicity** | 218 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 2.18, 10.15, 47.05, 218 mg/kg bw of the test material (FDA 71-57) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. The number of live litters, average implant sites per dam were similar for both test and control groups. The % partial resorptions and % complete resorption were increased only for the positive control group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses was similar for test and negative control groups. Also, there were no dead fetuses in either the test or negative control groups. The positive control group did show dead fetuses (3) and dams with more than one dead fetus. The positive control group also exhibited a decreased number of live fetuses and decreased average fetal weight compared to those for the negative control. Skeletal examination of sternebrae, vertebrae, skull, ribs, extremities, and soft tissues showed no significant differences between test and negative control groups. The positive control group showed a significant increase in incidence of missing sternebrae. Likewise, the positive control exhibited an increase in the incidence of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure. Visceral examination failed to reveal any evidence of abnormalities in either negative control or test groups. In the positive control group, meningoencephalocele and spina bifida were reported. |
| **Conclusion remarks** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 218 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restrictions. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973b) Teratologic evaluation of FDA 71-57 in rats. Contract No. FDA 71-260. Unpublished Report. |

rats. Contract No. FDA 71-260. Unpublished Report.

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Teratology study |
| **GLP** | Pre GLP |
| **Year** | 1973 |
| **Species/strain** | Hamster/female golden |
| **Sex** | Female |
| **Route of Administration** | Gavage |
| **Duration of Test** | 5 days |
| **Doses/concentration Levels** | 0(control), 4.05, 21.15, 98.2, or 405 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 10 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female hamsters were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated young adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, groups (19-23/group) of pregnant females were given 0, 64.05, 21.15, 98.2, or 405 mg/kg bw of the test material (FDA 71-57) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, % of live and % partial live resorptions, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The |

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| | remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects. |
| **NOAEL(NOEL) maternal toxicity** | 405 mg/kg bw/day |
| **NOAEL (NOEL) developmental toxicity** | 405 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 4.05, 21.15, 98.2, or 405 mg/kg bw of the test material (FDA 71-57) |
| **Maternal data with dose level** | Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses was similar for test and control groups. There was one dead fetus in the negative control and the 4.05 and 21.15 dose groups, but none in the higher dose groups. There were 18 dead fetuses in the positive control group. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and control animals. An increased incidence of incomplete ossification of the vertebrae was reported in the two mid-dose groups but not in the highest (405 mg/kg bw) group. Visceral examination of tissues failed to reveal any evidence of significant abnormalities at any dose level. |
| **Conclusion remarks** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 405 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restrictions. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973c) Teratologic evaluation of FDA 71-57 in hamsters. Contract No. FDA 71-260. Unpublished report. |

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| **Substance Name** | 4-*tert*-Butylcyclohexanol |
| **CAS No.** | 98-52-2 |
| **Remarks for Substance** | Data are for isomeric alcohol 2-Isopropyl-5-methylcyclohexanol (dl-menthol) |
| **Test Type** | Teratology study |
| **GLP** | Pre GLP |
| **Year** | 1973 |

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| **Species/strain** | Rabbit/virgin, adult, Dutch belted |
| **Sex** | Female |
| **Route of Administration** | Gavage |
| **Duration of Test** | 13 days |
| **Doses/concentration Levels** | 0(control), 4.25, 19.75, 91.7, 425 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin |
| **Exposure Period** | Days 6 to 18 of gestation |
| **Frequency of Treatment** | Daily |
| **Control Group and Treatment** | Control group received corn oil vehicle (10 ml//kg); Positive control received 2.5 mg/kg bw/day of 6-aminonictineamide in corn oil on Day 9 |
| **Remarks for Test Conditions** | Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female rabbits were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. On day 0, does were given an injection of 0.4 l of human chorionic gonadotropin (400 IU). Three hours later, each doe was artificially inseminated with 0.3 ml of semen from a buck using approximately 20 x 10e6 motile sperm. Beginning on Day 6 and continuing daily through Day 18 of gestation, groups (11-14/group) pregnant females were given 0, 4.25, 19.75, 91.7, 425 mg/kg bw of the test material (FDA 71-57) by gavage in corn oil. A positive control group received 2.5 mg/kg bw/day of 6-aminonicotinamide. Body weights were recorded on days 0, 6, 8, 12, 18, and 29 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 29 all dams were subjected to Caesarian section and the number of corpora lutea, implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. All live fetuses were placed in an incubator for 24 hours and evaluated for survival. All surviving pups were sacrificed and subjected to detailed visceral examination at 10x magnification. All fetuses were cleared with KOH, stained with alizarin red S dye, and examined for skeletal defects. |
| **NOAEL(NOEL) maternal toxicity** | 425 mg/kg bw/day |
| **NOAEL (NOEL) developmental toxicity** | 425 mg/kg bw/day |
| **Actual dose received by dose level and sex** | 0, 4.25, 19.75, 91.7, 425 mg/kg bw of the test material (FDA 71-57) |
| **Maternal data with dose level** | Survival of dams at term was similar for test, positive, and negative control groups. Daily clinical observation and measurement of body weight gain failed to show any |

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| | differences between control and test groups of female rabbits. The number pregnant and % pregnancy were similar for all dose and control groups. One to four pregnant female died in both control groups and in the four test groups. There was no dose response relationship for mortality in the test groups. There was no statistical difference in the number of live litters, corpora lutea, implantation sites, or resorption sites between the negative control group and any test group. |
| **Fetal Data with Dose Level** | The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses was similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Except for positive control group, skeletal examination of sternebrae and vertebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and untreated control group. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and the untreated control group The positive 6-aminonicotinaminde-treated control group showed increases in incidence of fused and split ribs. Visceral examination failed to reveal any evidence of abnormalities in either negative control or test groups. In the positive control group, medial rotation of the hind limb and anopia were reported in pups from 7 of the 11 litters. |
| **Conclusion remarks** | There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 425 mg/kg bw/day of test material. |
| **Data Qualities Reliabilities** | Reliability code 2. Reliable with restrictions. |
| **Remarks for Data Reliability** | Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles. |
| **References** | Morgareidge K. (1973d) Teratologic evaluation of FDA 71-57 in rabbits. Contract No. FDA 71-260. Unpublished report. |